DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

GILL DEVELOPMENT, GROWTH, AND RESPIRATION OF THE FLOUNDER *PLATICHTHYS FLESUS* *(L.)*

BY

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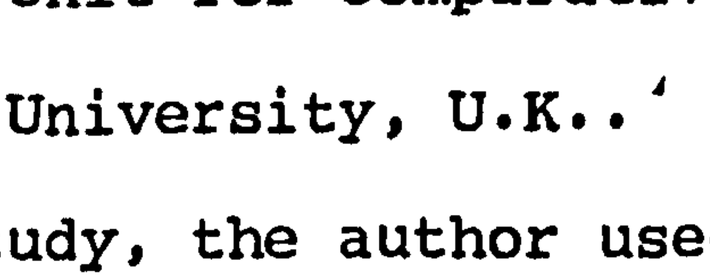
MEMORANDUM

The data and research for this thesis have been composed by the author, with exception of acknowledgement to references. The work was carried out under the supervision of Professor G.M. Hughes in the Research Unit for Comparative Animal Respiration, Bristol University, U.K.. During the period of this study, the author used the facilities of the Marine Biological Association Laboratory, Plymouth and the Institute of Marine Environmental Research, Plymouth.



Noori K. Al-Kadhomiy

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TO MY FAMILY AND

THE MEMORY OF

MY BELOVED BROTHER RASHEED

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a) 1982 Gill morphometry and respiration during development of flounder, Platichthys flesus (L.).

Society for Expirmental Biology, Leiden Conference, March-April, 1982.

b) 1983 Dimensions and micro-circulation of the gills of flounder, Platichthys flesus (L.).

Society for Experimental Biology, Bristol Conference, January, 1983

c) 1984 Vascular pathways in the gill filaments of the flounder, Platichthys flesus (L.).

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S U M M A R Y

SUMMARY

The relationship between oxygen uptake rate, temperature and activity have been studied for 0-group of flounder, Platichthys flesus, L. The data obtained may be helpful in evaluating the capability of this species to withstand the hypoxic water of the estuaries during low tide.

There are differences in gross morphology of the gills on the two sides of a fish following metamorphosis which are not present before metamorphosis. Relationships between gill dimensions and body weight were analysed using the relationship $Y = aW^b$ and suggested two-component curves for P. flesus. Changes in slope (b) for gill area/body weight relationships also occur at metamorphosis and on both sides of the fish. There is a significant decrease in growth of gill dimensions following metamorphosis. The lower side has fewer gill filaments and a smaller total gill area than those on the upper side. This reduction may be associated with a reduction in ventilatory currents which pass through the gills of the lower side.

The change in slope values of gill dimensions during development may be related to:

1. A change from cutaneous to gill respiration
2. Adoption of a demersal habit with corresponding reduction in activity and metabolic requirements

Before metamorphosis opercular cavities and opercular bones are symmetrical, but after metamorphosis they are asymmetrical. These changes possibly reflect the changing shape of the flounder, and the pressure of the water column in addition to body weight on the lower side of the fish.

Before metamorphosis, similar volumes of water are expelled through the opercular openings of both sides but after metamorphosis the water is expelled only through the upper opercular opening when the fish is resting.

Studies on micro-circulation of the gills suggest the presence of a separate filtration unit in the central venous sinus of the gill filaments.

Development, differentiation, functioning of the respiratory organs and cardiovascular system are discussed. Exponential relationships between heart beat frequency, opercular pumping frequency, heart mass, and body length relating to body weight are also discussed.

C H A P T E R 1

GENERAL INTRODUCTION

Chapter 1. GENERAL INTRODUCTION

Fish inhabit all three (fresh, estuarine and marine waters) parts of the hydrosphere. All the three water bodies are different in their physico-chemical characteristics. In all these environments, however, fish extract oxygen for their total metabolic activities by means of their gills. Fish gills are designed to perform respiratory and haemodynamic functions efficiently. The gills are so fascinating a subject for fish biologists that they have launched multi-disciplinary investigations to understand their structure and modus operandi.

1.1 MORPHOLOGY, MICROANATOMY AND FINE STRUCTURE OF FISH GILLS

The gross and microanatomy of gills have been studied by numerous workers in the field of fish respiratory biology in the past, of which Duvernoy (1839), Reiss (1881), Bietrix (1895), Plehn (1901), Faussek (1902), deserve special mention.

Duvernoy (1839), as quoted by Bijtel (1949), concentrated on the muscles concerned in the movement of the gill filaments. Reiss (1881) studied the microanatomy of the gill filaments of Esox lucius, Perca fluviatilis, Salmo salar and found that gill filaments are movable with the help of abductor and adductor muscles of the

constrictor branchialis. Bietrix (1894) first recognised the typical cell type separating the epithelial layers of the secondary lamellae, and termed them 'cellules en forme de piliers' or later 'cellules en pilaster' (Bietrix, 1895a). Plehn (1901) and Faussek (1902) designated the pillar cell as 'Pilaster Cells', "Zellen der Gefasschicht" and "Stuzellen" respectively. Goodrich (1930) presented an extensive report on the structure of fish gills. The presence of acidophil and non-mucoid glandular cells near the base of the secondary lamellae has been reported by Keys and Willmer (1932) in the gills of Anguilla vulgaris, Conger vulgaris, Salmo salar, Pleuronectes platessa, Cottus bubalis, Gobio fluviatilis and Leuciscus vulgaris and they assigned ionic regulatory functions to these cells and called them "Chloride cells".

Bevelander (1934, 1935, 1936) differentiated three types and seven sub-types of specialised branchial cells in the gill epithelium of cyclostomes, Elasmobranchs and Teleostean fish species. Similar cells have also been reported by Liu (1942) from the gill epithelium of a freshwater fish, Macropodus opercularis. He also reported hypertrophy of glandular cells acclimatised to increased salt concentration. Copeland (1948, 1950) also reported

chloride cells from the gill epithelium of Fundulus hetroclitus with special reference to extra renal ionic regulation. Pettengill and Copeland (1948) described the alkaline phosphatase activity of the chloride cells of Fundulus hetroclitus with their osmotic behaviour.

Vickers (1961) described in detail the functional organisation of the chloride cells from the gills of European eels and teleostean fish respectively. Philpott (1961, 1962, 1963 and 1965) presented an extensive account of the ultrastructure of the chloride cells of fish with special reference to their osmoregulatory function. An electron microscopical study on the chloride cells was also made by Threadgold and Houston (1961) from the gills of Salmo salar. Munshi (1960, 1964) differentiated mucous gland, acidophil granular cells, the goblet cells, the basophil and the acidophil mass cells from the gill epithelium of Indian freshwater teleostean fish with the help of various histological and histochemical methods.

Hughes and Grimstone (1965) described the ultrastructure of the secondary lamellae of the gills of Gadus pollachius. Newstead (1967) presented his findings on the ultrastructure of the respiratory

lamellae of teleostean gills. Munshi and Singh (1968) studied in detail the morphology, histology and microcirculatory system of the gills of some freshwater fish species. Singh and Munshi (1968) illustrated the cytology and cytochemistry of the granular cells found in the gills of certain fresh-water fish with special reference to their chloride regulation. Hughes and Munshi (1968) presented the finer structure of the gills of an Indian climbing perch, Anabas testudineus. Muir and Kendall (1968) detailed the structural modifications in the gills of tunas and some other oceanic fish. Hughes and Morgan (1973) studied the structure of gills in relation to their respiratory function. Wright (1973, 1974) described in detail the ultra-structure of the gills of the elasmobranch (Scyliorhinus canicula) and lung fish (Lepidosiren paradoxa) respectively. Olson and Fromm (1973), Kimura and Kudo (1979), Hughes (1979) and Kendall and Dale (1979) studied in detail the gills of the rainbow trout, Salmo gairdneri with special reference to their ultrastructure. Hughes and Munshi (1979) showed in detail the fine structure of the gills of Indian air-breathing fish. Dunel and Laurent (1980) described the ultrastructure of the gills of the sole, Solea solea. Hughes and Mittal (1980) have described the structure of the gills of Barbus sophor

with tertiary lamellae with the help of light and electron microscopy. Hughes (1981) presented his ideas on the past, present and future of fish gills in the scientific world.

1.2 MICROCIRCULATORY SYSTEM

Fish gills also provide an interesting architectural plan of the respiratory and haemodynamic systems. The vasculature of the gill filament is represented by two separate (the arterio-arterial and arterio-venous) pathways. The arterio-arterial vascular system in the gill filament receives blood from an afferent branchial artery, from this afferent filament artery blood passes through the secondary lamellar channels leading into the efferent filament artery and efferent branchial artery enabling oxygenated blood to perfuse the systemic pathways. The respiratory path of the blood flow through the gills is well established (Allis, 1912; Goodrich, 1930; Mott, 1950; 1951; Muller, 1839; Munshi and Singh, 1968; Muir, 1970).

The arterio-venous pathway in the gills has been a matter of dispute in the past (Reiss, 1881; Steen

and Kruysse, 1964; Richards and Fromm, 1969). But now it is well established that in teleosts this system is formed by a complex arrangement of blood channels and veins that directly return the blood to the heart bypassing the systemic circulation. Two experimental procedures are adopted to establish arterio-venous pathways in fish having different respiratory adaptations. In the first method semithin and ultrathin sections of gill filaments are prepared and the vascular pathways are traced with the help of light and electron microscopy (Steen and Kruysse, 1964; Vogel, Vogel and Kremers, 1973; Vogel, Vogel and Schlote, 1974; Vogel, Vogel and Pfautsch, 1976 and Vogel, 1978).

In the other method, vascular replicas of gills are obtained by perfusing resins through the blood vascular system of fish. Two types of resin are used for obtaining vascular replicas. Dunel and Laurent (1977), Laurent and Dunel (1976), Laurent, Delaney and Fishman (1978) have used Microfil to obtain vascular replicas of the gills. However, Boland and Olson (1979), Olson (1980, 1981) and Olson and Kent (1980) perfuse methyl methacrylate resin and catalyst to obtain vascular replicas. The detailed arterio-vascular pathway is traced with the help of scanning electron micrographs.

The present study reports findings on the morphology, microanatomy, fine structure, dimensions and micro-circulatory systems of the flounder, Platichthys flesus.

1.3 GILL DIMENSIONS

Fish are primarily water breathers using gills efficiently for oxygen uptake. The capability of gills depends on their effective surface area and water-blood diffusion distance. In recent years, attempts have been made to measure the dimensions of the respiratory organs of fish to assess their capability in oxygen uptake rate and carbon dioxide release. Earlier measurements of gill surfaces have been relatively few in number and general in treatment. Pütter (1909) presented his data on gill dimensions of four specimens of Scorpaena. However, the work of Price (1931) on the small-mouthed black bass, Micropterus dolomieu seems to be the first detailed study on the gill dimensions of a single species over its entire life cycle. But the methodology used in this study was not adequate enough to present good estimates of gill dimensions since the profiles and dimensions of secondary lamellae were not considered. Subsequently, various studies were made (e.g. Gray, 1954; Byczkowska-Smyk, 1957, 1958, 1959a,b, 1961,

1962) to evaluate the gill dimensions of various fish species until Hughes (1966) put forward a new method for the determination of gill surface area in fish. He considered various gill parameters (total filament length, frequency of secondary lamellae and area of secondary lamellae) which contribute to the total gill area and presented data on gill dimensions for fourteen species of British fish and Antarctic haemoglobin-less ice fish, Chaenocephalus aceratus. The data on total gill dimensions were obtained with the help of the equation:-

Total gill area = (2L/d) bl

where, L = total filament length,

$^{1}/_{d}$ = frequency of secondary lamellae per mm on one side of filament, and

bl = average bilateral surface area of a secondary lamella

Hughes (1966) believes that more active fish have larger gill area and better gaseous exchange capability in comparison to sluggish ones. This is due to greater filament length. With the help of his data on gill morphometry, he has pointed out that the resistance to flow relative to area is less in sluggish fish due to more widely spaced secondary lamellae. Muir and Hughes (1969) advocated a weighted method for the determination of gill dimension in fish. With the help of this method, they estimated the total gill

area of skipjack tuna (Katsuwonus pelamis), yellowfin tuna (Thunnus albacares) and bluefin tuna (Thunnus thynnus). Quantitative relationship was achieve between body weight and various gill parameters for these species.

For all three species, the slope of the regression line relating gill area to body weight was 0.85, a value close to that (0.81) obtained for oxygen consumption and body weight for a large number of teleost species (Winberg, 1956). Since then this method has been widely used for gill measurements (Hughes, 1970; 1972; Hughes and Gray, 1972; Ojha and Munshi, 1974; Hakim, Munshi and Hughes, 1978; Munshi, Ojha and Sinha, 1980; Niva, Ojha and Munshi, 1981).

Hughes and Morgan (1973) revised the weighting method for the detailed measurement of gill dimensions. They also pointed out the effect of different sampling methods on gill dimension measurements and suggested using corresponding regions for sampling the frequency and average bilateral surface area of the secondary lamellae on each of the selected filaments. Since then, the method with some modifications was used for the measurement of the oceanic sunfish, Mola mola (Adeney and Hughes, 1977), the Pacific deep-sea fish, Synaphobranchus affinis, Serrivomer sector, Gonostoma elongatum, Bathythphlops marionae, Barbourisia ruffa and Xyelacyba myersi

(Hughes and Iwai, 1978), the coelacanth, Latimeria chalumnae (Hughes, 1980). However, little is known of the effect of growth on gill dimensions of the larval and adult stages of fish (Morgan, 1971; De silva, 1974).

1.4 OXYGEN UPTAKE RATE (OXYGEN CONSUMPTION)

Metabolism of fish has been actively studied during the last 20 years, because of its great importance in comparative physiology (Hughes, 1974; 1976; Piiper,1972). Metabolic rate is generally determined by measuring oxygen consumption per unit weight in unit time. Metabolic rate determines the amount of energy expended in producing biomass and therefore, is also of importance in determining the role of the animals in community metabolism.

In general, animals are either oxygen regulators or conformers according to the dependency of their rate of oxygen consumption on oxygen concentration in the environment . Therefore, the provision of oxygen is one of the most pressing demands on the physiology of fish and is made more difficult by the physical properties of their respiratory medium.

Fish, like other cold-blooded animals, do not have a definite stable resting or basal metabolic rate. Consequently, metabolic rate of fish can only be measured by making repeated measurements under different conditions. Oxygen uptake rate can be affected by many factors (e.g. temperature, activity, body size, seasons, ...etc.) (Fry, 1957; 1971; Winberg, 1956; Brett, 1962; 1964; 1965; Beamish, 1965).

Three general methods have been used :

1. Measuring the depletion of oxygen in a closed respirometer.

2. Measuring the O_2 removed and rate of flow water through a chamber (continuous flow), and

3. Manometric methods using very small animals.

Fry (1957 in Brown) distinguishes three levels of oxygen consumption resting (standard), routine, and active. Standard $\dot{V}_{O_2}$ may be taken as O_2 uptake rate in the absence of spontaneous activity, whereas, routine $\dot{V}_{O_2}$ is the mean O_2 uptake of fish whose only activity is spontaneous.

1.4.1 Oxygen consumption in relation to body size

Resting metabolism of animals in relation to body size has received considerable attention for many years(Kleiber, 1932; Brody, 1945; Zeuthen, 1947; Winberg, 1956; Fry , 1957; Hemmingsen, 1960; Beamish, 1964; Brett, 1962; 1964; 1965), of the following relationship has been shown for all groups: oxygen consumption in unit time,

$$\dot{V}_{O_2} = aW^b$$

when the value of (b) varies between 0.67-0.80 depending on the species of animal concerned. Emphasis has tended to be given to studies at the interspecific level showing that values for resting $\dot{V}_{O_2}$ of different species fall on the straight line (slope = 0.75),when plotted logarithmically against body mass. It has become increasingly apparent that it is important to consider or even expect deviations from 0.75 not only in relation to resting metabolism but for other features of the

structure and function of respiratory systems (Hughes, 1984).

1.4.2 Oxygen consumption in relation to activity

Oxygen consumption is proportional to the activity of animal. Most experiments are carried out for measuring resting or basal metabolism. In most cases the values for standard V_{O_2} are obtained where the same species is kept for a long acclimation period in a respirometer in order to reduce activity.

Active metabolic rate in relation to body size does not necessarily follow a course parallel to line that for standard metabolic rate (Hughes, 1984). The difference between the active and standard metabolic rate has been termed by Fry (1947) "the scope for activity".

In mammals active $\dot{V}_{O_2}$ has the same slope as standard $\dot{V}_{O_2}$ (Weibel and Taylor, 1981), whereas, in several fish active $\dot{V}_{O_2}$ has a greater slope than standard V_{O_2} (Brett, 1964; Brett and Glass, 1973; Duthie, 1980; Graham, 1982). In discussing this difference Hughes (1984), has concluded that with increase in size, either P_{O_2}/t (t = barrier thickness) must decrease or maximum $\dot{V}_{O_2}$ must increase more rapidly than standard $\dot{V}_{O_2}$. It seems possible that the first alternative is found in mammals, whereas, the second is more typical of fish.

1.4.3 Oxygen consumption in relation to temperature

Variations in environmental temperature impose a variety of stresses upon teleost fish, not least of which those associated with respiratory requirements. The effect of temperature upon oxygen solubility is such that the teleost exposed to elevated temperature conditions has a heightened oxygen demand in an environment which has become relatively hypoxic. Oxygen consumption in relation to temperature can often be described for fish in terms of a parabolic function with two constants having the general form:

$$\dot{V}_{O_2} = K.T^{K_1}$$

or its arithmetic equivalent

$$V_{O_2} = K + K_1.T,$$

i.e. the well known Belehradek and Krogh metabolism-temperature curves. The second constant, K_1, which represents the Van't Hoff Q_{10} for overall oxidative metabolism, may have value ranging from about 2 to 10 depending upon the species and temperature zone under consideration (Winberg, 1956; Fry, 1957).

As reference to recent reviews will illustrate (e.g. Randall, 1970; Johanson, 1971; Hughes and Morgan, 1973; Jones and Randall, 1978) the ciculation, respiration, ventilation and othe aspects are based to a large extent on experiments with rainbow trout, Salmo gairdneri in freshwater. However, the results

1.5 GILL VENTILATION

Ventilation is one of the important physiological factors which influence the oxygen uptake rate capability of respiratory organs. In teleost, gill ventilation is under the influence of buccal pressure and opercular suction pumps (Hughes and Shelton, 1962). The visceral skeleton under the influence of co-ordinated movements of cranial muscles operate the respiratory pumps.

The processes involved in gill ventilation have attracted the attention of many fish biologists in the past (Baglioni, 1907; 1910; Willem, 1931; 1940; 1947; Woskoboinikoff, 1932; Van Dobben, 1935; Kirchhoff. 1958). These studies were based mainly upon anatomical details and therefore, failed to elucidate exact mechanism of gill ventilation. With the advent of electrophysiological technique, however, the mechnics of gill ventilation have been demonstrated in many fish species (Hughes and Shelton, 1957; 1958; 1962; Ballintijn and Hughes, 1965; Hughes and Ballintijn, 1965; Hughes, 1973). Little, however, is known of the morphological adaptations connected with gill ventilation of flatfish (Yazdani and Alexander, 1967; Yazdani, 1976). Observations are reported in this study to describe morphological and physiological adaptations associated with gill ventilation in the flounder, Platichthys flesus.

1.6 EMBRYONIC DEVELOPMENT

Fish eggs have great diversity in their morphological features and the content of the yolk. Quantity of yolk present in the eggs determines their future development. The larval stages of Platichthys flesus are transparent, symmetrical with one eye on each side of the head and swim in a vertical position like other "round fish". In adult stages, fish are recognised from their strong laterally compressed body form.

Development, differentiation and functioning of the respiratory organs and cardiovascular system of the flounders is not well known. The present study also reports various developmental stages of Platichthys flesus with special reference to the respiratory and cardiovascular system.

The aim of this study is an attempts to elucidate the following points:

1. There has been little or no study concerning the effect of the metamorphic change in flatfish on the weight-oxygen uptake relationship of 0-group fish of flounder. Chapter 3 is an attempt to study the quantitative relationship between body weight and temperature on standard oxygen uptake rate in 0-group

of the flounder, Platichthys flesus.

2. Little is known on the micro-circulatory systems pathway in fish gills (Reiss, 1881; Steen and kruysse, 1964; Richards and Fromm, 1969; Vogel, et al, 1973; 1974; 1976; Laurent and Dunel, 1976; 1980). Chapter 4 is concerned to elucidate the functional organization of the gills of the flounder, Platichthys flesus.

3. Very few studies have been carried out on the development of gill morphometry of flatfish (De Silva, 1974). Chapter 5 is an endeavour to elucidate the effect of growth on the various gill parameters of pre- and post-metamorphic stages of the flounder, Platichthys flesus.

4. In Chapter 6 the study is concerned with the development, differentiation and functioning of the respiratory organs and cardiovascular systems. Heart beat frequency, opercular pumping frequency, heart mass, and length-weight relationship are discussed.

5. Chapter 7 is an attempt to describe the morphological adaptations which are associated

with gill ventilation, opercular cavities, and opercular bones of the flounder, Platichthys flesus.

C H A P T E R 2

MATERIALS AND METHODS

CHAPTER 2 MATERIALS AND METHODS

2.1 THE FISH

The flounder, Platichthys flesus L., is one of the flatfish species, belonging to the order Heterostomata, family Pleuronectidae. During larval stages, these fish are transparent and symmetrical, with one eye on each side of the head, and they swim in a vertical position like other "round fish". As adults, such fish are recognised by their strong, laterally compressed body form. This flattened shape means that both eyes are on the upper side, which is also coloured. The lower or blind side becomes white as they become adult and settle down on the bottom. They do not descend to any considerable depth and swim with undulating motion of their body. They are mostly abundant in areas of rather low salinity, such as the Baltic Sea and around the N.E. coast of Scotland. They are an important food fish and are a commercially important group in most European countries. These flounders migrate upstream in summer, but they move off shore to their spawning grounds which are situated in deeper regions (Lythgoe and Lythgoe, 1971). They are found in the tidal zone to a depth of 25m (Muus and Dahlstom, 1975) or more than 50m (Lythgoe and Lythgoe, 1971). Summers (1979) has even found them in totally fresh water environments. The flounder is thus exposed to considerable variation in ambient

physico-chemical conditions including changes in temperature, salinity, dissolved respiratory gases and lower pH ranges (Weber and De Wilde, 1975).

2.2 COLLECTION SPOTS AND MAINTENANCE

The experiments on their artificial breeding and maintenance with algae Phacodactylum triornulum (Bohlin), Rotifera, Branchionus plicatilis and Artemia, Artemia salina, were carried out at the Marine Biological Association Laboratories, Plymouth, U.K. in 1980 and 1981, during the period March to June. The mature males and females were captured by trawls off Plymouth in the Hamoaze and Tamar Estuaries. The fish were placed in concrete tanks with a capacity of about 5x1x1 metre, which were supplied with running aerated sea water at 12-13^{o}C. The fish were fed twice weekly with shrimps, Artemia, Tubifex and Bloodworms.

2.3 ARTIFICIAL BREEDING

Ripe eggs from mature females were taken by squeezing gently the belly directly into a crystalizing dish containing a small amount of filtered, sterilized sea water (depth 1-3 cm). Sperms were taken from mature males by stripping the testis and using a pipette and the sperms were mixed in a small amount of filtered, sterilized sea water in a watch glass. This

was tipped into the crystalizing dish and agitated and shaken gently. The contents were kept in a constant temperature room at 10°C; after about 30 minutes more, sea water was added to dilute the contents.

The fertilized eggs floated to the surface of the water, while the unfertilized and dead eggs sank down to the bottom of the container. The fertilized eggs were washed and removed to further fresh sea water using a tea strainer. Deformed, abnormal or dead eggs were removed with the help of a wide-necked pipette to avoid disturbing or damaging the other eggs. The water was changed daily until the feeding behaviour started. Occasionally the fertilized eggs were supplied by MBA-Laboratories using the following procedure for transporting the eggs to RUCAR-Laboratories:-

(i) Fertilized eggs with sea water were placed in polythene bags and oxygen was pumped on the top of the eggs, which were sealed completely from the outer atmosphere.

(ii) The polythene bags were placed in a thermos, surrounded by ice, and eventually covered and transferred the same day to RUCAR, Bristol.

Sea water of salinity 35% was prepared artificially from the following materials:-

NaCl	.....	0.41256 M/kg sol.
Na_2SO_4	.....	0.02024 " "
NaF	.....	0.00007 " "
KCl	.....	0.01021 " "
MgCl	.....	0.05292 " "
$CaCl_2$	.....	0.01028 " "

2.4 REARING OF FISH EMBRYOS

Fertilized eggs and fish embryos were reared in laboratories under a controlled temperature, light and oxygen supply. Figure 1 shows the system which has been used for rearing fish. Each incubator tray was placed in a plastic container, through which tap water was passed at constant temperature controlled by a thermostat (fluctuation was $\pm$ 0.5°C at all temperatures). Water was pumped from a cooling system to a constant head, which fed two containers (Fig.1). The incubation tray water was aerated continuously and each tray has covered with a polythene sheet.The water was renewed daily until the feeding behaviour started when the whole yolk sac is absorbed; then the fish were transferred to larger plastic containers which were continuously aerated and placed in front of fluorescent lights at a constant room temperature.

FIGURE 1

Diagram showing the system which is used for fertilizing and rearing fertilized eggs until feeding behaviour started.

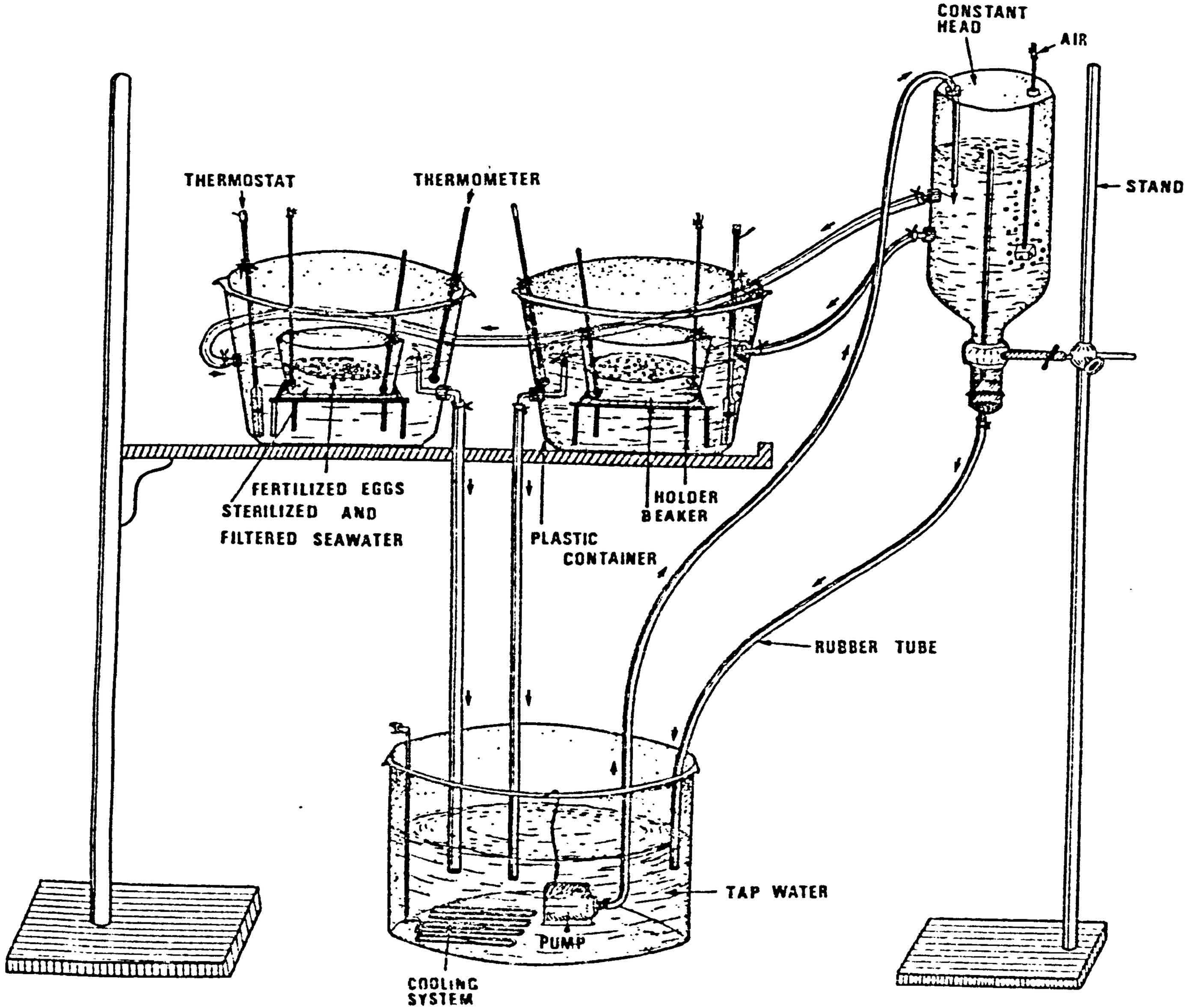

CONSTANT HEAD
AIR
STAND
THERMOSTAT
THERMOMETER
FERTILIZED EGGS
STERILIZED AND
FILTERED SEAWATER
HOLDER BEAKER
PLASTIC CONTAINER
RUBBER TUBE
TAP WATER
PUMP
COOLING SYSTEM

PLATE 1

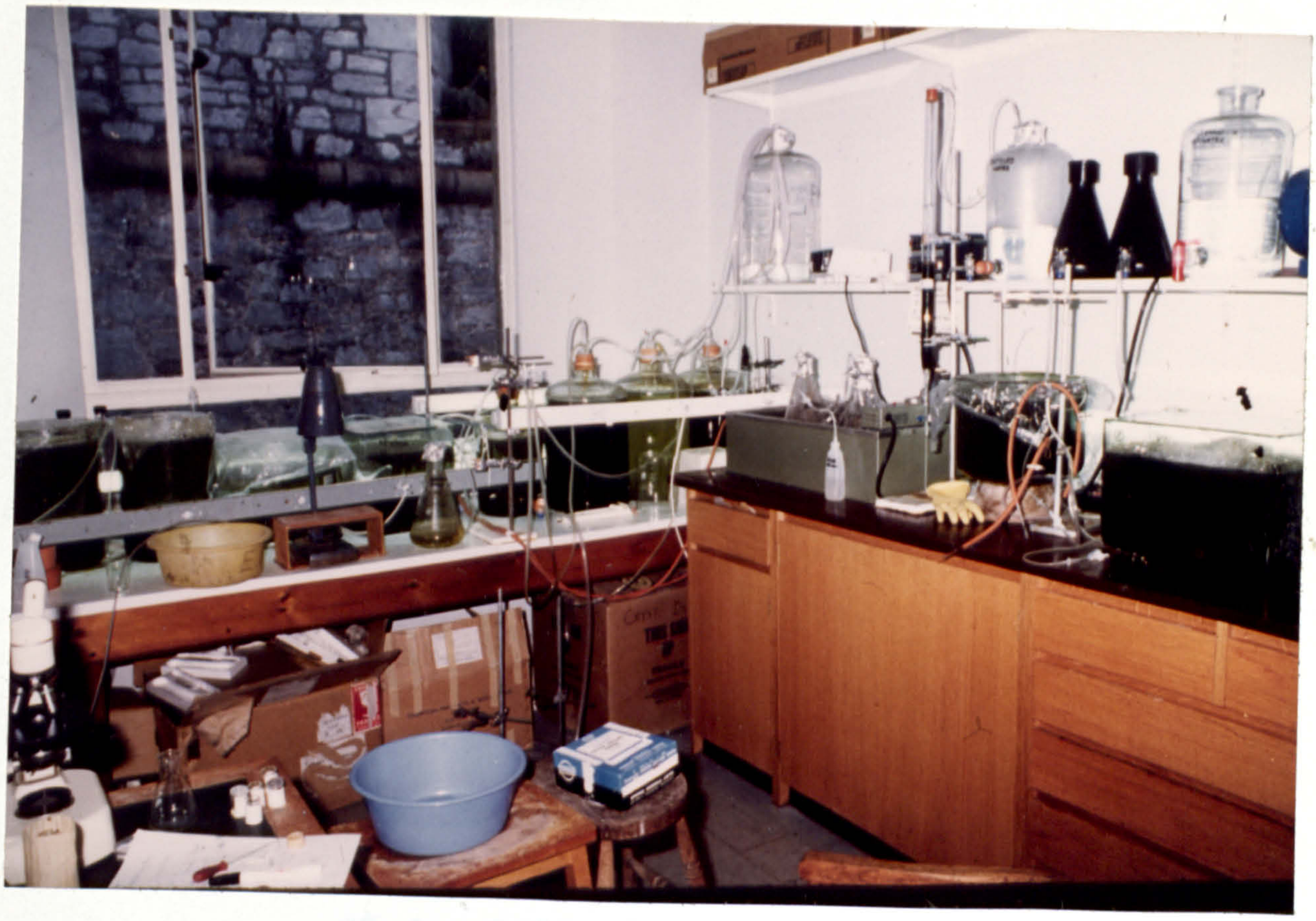
a: Photograph showing Phyto-zooplankton culture at Laboratory

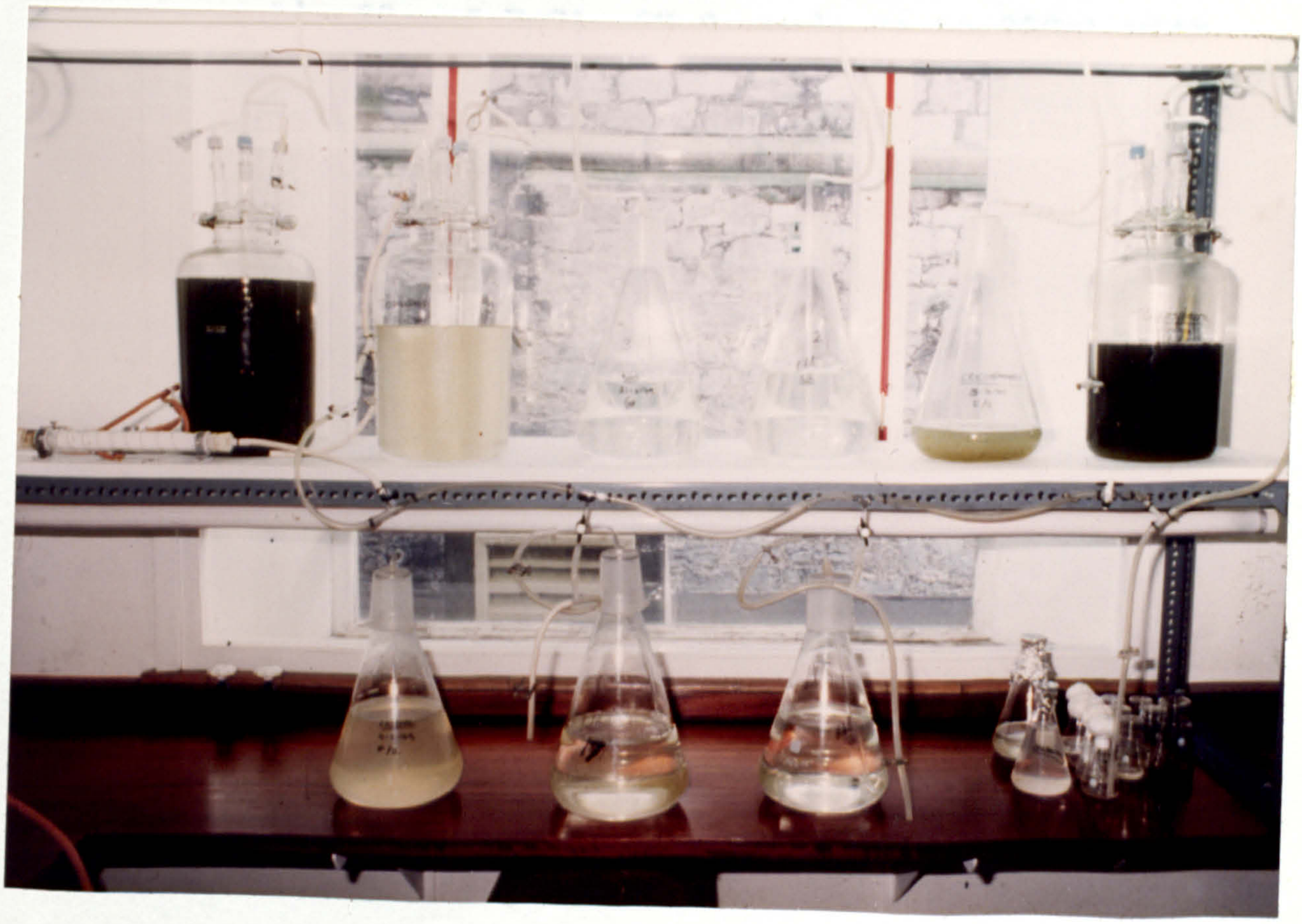
b. Photograph showing stock cultures of:-

a) green algae, Phacodactylum triornulum,

b) rotifera, Brachionus plicatilis, and

c) artemia nuplii, Artemia salina.





Algal Culture Medium

(i) Major Nutrients

a) Nitrate - 25g of KNO_3 made up to 250ml with distilled water (used 1.0ml/litre).

b) Phosphate - 3.4g of KH_2PO_4 made up to 250ml with distilled water (used 1.0ml/litre).

(ii) Minor Trace Metals

a) 30mg of $ZnSO_4.H_2O$, 25mg $CuSO_4.5H_2O$, 25.4mg $CoCl_2.6H_2O$.

b) 50g of $FeCl_3.6H_2O$, made up to 250ml with distilled water. 17g of $MnCl_2$ made up to 250ml with distilled water.

c) 25mg of $Na_2MnO_4.2H_2O$ made up to 250ml with distilled water.

d) 50g of E.D.T.A. (Na salt) made up to 1 litre with distilled water.

Working Mixture of the Trace Metals

200ml of d) and 5ml each of a), b) and c) were added to about 800ml of H_2O. pH was adjusted with diluted

NaOH to pH 7.4. The solution was made up to 1 litre with distilled water. (Used 1.0 ml/litre.)

(iii) Vitamins

a) B_{12} - 10.0mg of B_{12} was dissolved in 100ml distilled water and was frozen immediately. Before use, the solution was brought to room temperature and 1.0ml was diluted to 50ml with distilled water. (1.0ml/litre was used.)

b) Biotin - 10.0mg of Biotin was dissolved in 100ml of distilled water and frozen immediately. Prior to use, 1.0ml of the solution was diluted to 50ml with distilled water. The solution was refrozen. Excess of diluted solution was not used again (1.0ml/litre was used).

c) Dichloroisosyanuric acid (sodium salt) "used instead of Thymine Hydrochloride (B_1)".

100mg of Dichloroisosyanuric acid was dissolved in 100ml of distilled water and stored in a deep freeze. Prior to use, 1.0ml of the solution was diluted with distilled water and was refrozen. Excess of dilute

solution was not used again (concentration used was 1.0ml/litre).

1ml each of B_{12}, Biotin and Dichloroisosyanuric acid were diluted with distilled water to 37.5ml.

The medium normally was prepared in 20 litre carboys. 20ml each of nitrate, phosphate, metal mixture and 15ml of diluted vitamin mixture solutions were added to the 20 litre carboys. The mixed solution was autoclaved at 15lb for 15 minutes in small bottles.

2.6 PREPARATIONS OF SPECIMENS FOR LIGHT MICROSCOPY

Specimens for light microscopy were fixed according to their sizes. A few gill filaments (primary lamellae) were dissected carefully and fixed for large specimens; the gill arch for intermediate sizes; the whole fish was fixed after the removal of both opercula from very young specimens.

Fixation

Specimens of different sizes were fixed in the following fixatives:-

(i) Bouin's fixative (Bouin, 1857) for 12 to 24 hrs.

Picric acid (saturated aqueous sea water) 75ml

Formalin	25ml
Glacial acetic acid	5ml

(ii) Heidenhains Susa fixative (Susa, 1918) for 3 to 4 hours.

Mercuric chloride	45g
Distilled water	300ml
Sodium chloride	5g
Trichloracetic acid	20g
Glacial acetic acid	40ml

Mercuric chloride was dissolved in distilled water using heat. When the mixture became cold, other reagents were added to form the stock solution of the fixative. 20ml of formalin was added to 80ml of stock solution to make up 100ml of fixative.

Dehydration

Specimens after fixation were passed through the following concentrations of ethyl alcohol for dehydration:-

70% alcohol - washed several times until no yellow colour escaped to the alcohol. Usually three hours depending on the size of the specimens.

95% alcohol - two hours, depending on size of specimens.

100% alcohol- two hours, depending on the size of specimens.

Clearing

Cedarwood oil was used as a clearing agent and then rinsed in Benzene.

Embedding at 60°C

56°C Paraffin wax	-	2 hours
56°C Paraffin wax	-	2 hours
56°C Paraffin wax	-	4 hours

Staining

For staining, the following reagents were used:-

(i) 1% aqueous acid fuchsin

(ii) Mallory's stain, which consists of the following components:-

Aniline blue	0.5g
Orange G	2.0g
Phosphotungstic acid	1.0g
Distilled water	100 ml

Staining procedure:

(i) After dewaxing, sections were bathed in distilled water and treated with Iodine to remove mercury and also to act as a mordant.

(ii) The sections were then washed in distilled water for five minutes.

(iii) The nuclei were stained in haematoxylin.

(iv) Nuclear differentiation was done with acid water for a few seconds and washed in tap water.

(v) After nuclear staining, the sections were treated with acid fuchsin for five minutes.

(vi) The slides were washed in distilled water for 30 minutes and rinsed again in distilled water.

(vii) The sections were again stained in Mallory's stain for 20 minutes and washed in distilled water for 2-5 minutes.

(viii) The sections were dehydrated in progressively increasing concentrations of ethyl alcohol as follows:-

50%	alcohol	2-3 minutes
70%	alcohol	2-3 minutes
95%	alcohol	2-3 minutes

100% alcohol ..... 2-3 minutes

100% alcohol ..... 2-3 minutes

(ix) The sections were cleared in Xylene for 1-2 minutes, mounted in D.P.X., covered by coverslip and dried.

2.7 PREPARATIONS OF SPECIMENS FOR ELECTRON MICROSCOPY

Fixation

Gill specimens were taken from different stages during development of the flounder and were fixed at 0-4°C for one hour (10 minutes followed by 50 minutes in fresh fixative) in one of the following fixatives:-

(i) 5% glutaraldehyde with collidine, buffered at pH 7.4 with marine teleost saline (Young, 1933).

(ii) 3% glutaraldehyde with 0.2M sucrose, buffered at pH 7.4 with 0.1M cacodylate buffer (Millonig, 1961).

Washing

After fixation, the specimens were washed in 0.1M cacodylate buffer six times for five minutes.

Postfixation

The specimens were post-fixed in 1% osmium tetroxide with 0.2M sucrose buffered at pH 7.4 with 0.1M cacodylate for 10 minutes, followed by 50 minutes in fresh fixative (Sabatini et al.,1963).

Dehydration

Specimens were dehydrated in ascending series of ethyl alcohols:-

30% alcohol	5-10 minutes at 0-4°C
30% alcohol	" " " "
50% alcohol	" " " "
50% alcohol	" " " "
70% alcohol	" " " "
70% alcohol	" " " "
90% alcohol	" " " "
90% alcohol	" " " "
100% alcohol	" " " "
100% alcohol	30 minutes at room temp.
100% alcohol	" " " " "

Embedding

Specimens were embedded as follows:-

acetone : araldite (3 : 1)	60 minutes
acetone : araldite (1 : 1)	60 minutes

acetone : araldite (1 : 3) ..... overnight
(approx. 18 hours)

Polymerisation

Araldite at 60°C for two days.

Semi-thin (1 to 2 um) and ultra-thin sections were obtained using LKB Ultratome III and stained in 1% Toluidine blue and 1% azure in 1% borax (Richardson, et al., 1960). The ultrathin sections were cut with a glass knife, mounted on carbon-coated grids, stained for 15 minutes with 2% aqueous uranyl acetate adjusted to pH 7.4 with 1N NaOH, washed in distilled water and counter-stained for 15 minutes with lead citrate (Reynolds, 1963). The sections were examined with EM 6G and Philips 300 electron microscopes, operated at 80KV.

C H A P T E R 3

OXYGEN UPTAKE IN RELATION TO BODY WEIGHT OF PREMETAMORPHIC (O-GROUP) STAGES OF *PLATICHTHYS FLESUS* (L.)

Chapter 3 : OXYGEN UPTAKE IN RELATION TO BODY WEIGHT OF PREMETAMORPHIC (O-GROUP) STAGE OF PLATICHTHYS FLESUS (L.)

3.1 INTRODUCTION

Metabolism can be measured by direct calorimetry which involves measurement of the heat production of an animal. It is not a practicable technique for fish, mainly because of the problem of measuring heat liberation in water with its large specific heat and consequent insensitivity to temperature change, and the relatively low metabolic rate of ectotherms (Brett, 1962). Hardy (1963) discussed various direct calorimetric methods. Indirect calorimetric methods are often used to estimate energy metabolism. These involve the measurement of oxygen consumption. Oxygen consumption can be used as a practical measure of metabolic rate because the amount of heat produced for each unit of oxygen used remains nearly constant, irrespective of whether fat, carbohydrate or protein is oxidised (Schmidt-Nielsen, 1979).

Three levels of metabolism in fish are defined by Fry (1957). These are standard (resting, basal), routine and active. Standard metabolism (which means standard oxygen consumption in this study) is the minimum energy cost when the animal is at rest in a thermoneutral (or temperature acclimated) environment in the post-absorptive condition (Brett, 1962).

Standard oxygen consumption, therefore, is an approximation of the minimum metabolic rate of the intact organism. In spontaneously active fish, it is frequently determined by simultaneously measuring a controlled activity parameter such as swimming speed and oxygen consumption and extrapolating the relationship back to zero activity (Fry and Hochachka, 1970). Routine metabolism refers to the metabolic rate when movements are restricted and the fish is protected from outside stimuli but is free to move and does so occasionally (Hoar and Randall, 1978). Active metabolism is defined as the maximum rate consistent with highest continued level of activity (Brett, 1964). The difference between the active and standard rates of metabolism is called "the scope for activity" (Fry, 1947). The rate of oxygen uptake is influenced by various factors, viz. P_{O_2}, P_{CO_2}, pH, temperature, salinity of water, body size, activity, sexual maturity and season etc. (Beamish, 1964; Brett, 1962; Fry, 1971).

3.1.1 OXYGEN CONSUMPTION IN RELATION TO BODY SIZE

Body size is one of the important factors which influences the metabolic rate of fish, which in turn increases steadily with body weight but it does not

increase in direct proportion to body weight. Typically basal or standard metabolic rate is related to body weight according to the following equations:

$$\dot{V}_{O_2} = aW^b \quad \text{.....(1)}$$

where $\dot{V}_{O_2}$ is the total metabolic rate, W is body weight and a and b are respectively the intercept and the regression coefficient. If b is equal to 1, the equation will be reduced to:

$$\dot{V}_{O_2} = aW$$

However, b is usually less than 1 and therefore weight specific metabolic rate decreases with increase in body weight.

The relationship between weight specific metabolic rate and body weight is usually expressed by the equation:

$$\frac{\dot{V}_{O_2}}{W}2 = aW^{(b-1)} \quad \text{.....(2)}$$

When the logarithms of both sides of the equations ($\dot{V}_{O_2} = aW^b$ and $\frac{\dot{V}_{O_2}}{W}2 = aW^{b-1}$) are taken, the relationships between oxygen consumption and body weight per unit time and per unit body weight respectively are now expressed by:

$$\text{Log } \dot{V}_{O_2} = \text{Log } a + b \text{ Log } W \text{ .....(3)}$$

and

$$\text{Log } \frac{\dot{V}_{O_2}}{W} = \text{Log } a + (b-1) \text{ Log } W \text{ .....(4)}$$

Ever since the relationship between metabolic rate and body weight was discovered, much interest has centred on explaining it. Morgulis (1914), and Rubner (1924) have reported deviations in the slope of regression lines relating logarithms of metabolic rate and body weight. In Fundulus parvipinnis the slope of the regression line relating oxygen uptake per unit time and body weight has been reported to be between 0.5 and 0.6 (Keys, 1931; Wells, 1935). However, Smith (1935) has reported a higher value (b = 0.9) for the African lungfish. Müller (1942) reported a slope of 2/3 for Lebistes reticulatus. Zeuthen's (1947) data for various fish species gave a slope of 0.78. Higher slope value (0.85) has been reported for both arctic and tropical fish (Scholander et al. 1953). Similar value (0.85) has been recorded for the speckled trout, Salvelinus fontinalis (Job, 1955).

In recent years also, similar studies have been extended to various fish species to present quantitative relationship between the two parameters (Brett, 1965; Kamler, 1972; Hughes, 1977, 1978; Munshi et al. 1978).

3.1.2 EFFECT OF TEMPERATURE ON STANDARD OXYGEN CONSUMPTION

In general, biological processes are based on chemical reactions which are profoundly influenced by temperature (Bullock, 1955). This may limit the geographic distribution of animals (Prosser and Brown, 1961) and at the same time determine their rate of activity (Fry, 1971). The effect of temperature can be expressed most simply in terms of the Q_{10} value. The Q_{10} for a particular reaction is thus an expression of the increase in its rate for a $10^{o}C$ increase in temperature. For most biological reactions, the Q_{10} is between 2 and 3.

Krogh (1916) expressed the relationship between temperature and standard oxygen consumption as the "normal curve". It is used as an indicator of the relationship between standard oxygen consumption and temperature (Precht et al. 1973). The curve is characterised by a reduction in Q_{10} value as

temperature increases. Winberg (1956), in his extensive review, suggested that the response of the metabolism of fish to temperature more or less fitted the normal curve. However, more recently the universality of the curve has been questioned. Fry (1971) gives a constant Q_{10} of 2 for goldfish, the species on which the normal curve was initially determined. Brett (1964) and Edwards et al.(1969) found that the Q_{10} increase with increasing temperatures for a number of species. The relationship between oxygen uptake rate and body weight at different temperatures has been established for a number of flatfish species (e.g. Hickman, 1959; Edwards et al. 1969, 1971; Duthie, 1982; Duthie and Houlihan, 1982).

3.1.3 EFFECT OF HANDLING ON STANDARD OXYGEN CONSUMPTION

Many studies have shown that after transfer from a holding tank to a respirometer, the oxygen consumption of fish is initially high and returns to a steady level thereafter (Winberg, 1956; Saunders, 1962; Fry, 1971; Holeton, 1974). This increase has been attributed to an increase in spontaneous activity, or recovery from oxygen debt (Holeton, 1974). Thus in the present study, a sufficient period of recovery from handling was allowed before accurate determinations were recorded.

Most of the quantitative analyses between body weight and temperature on oxygen uptake rate have been made on fish which maintain the same body contour throughout their whole life history. However, there has been little or no study concerning the effect of the metamorphic change in flatfish on the weight-oxygen uptake rate relationship.

The present chapter is an attempt to throw light on the quantitative relationship between body weight and temperature on standard oxygen consumption rate in O-group of the flounder, Platichthys flesus.

3.2 MATERIALS AND METHODS

In this study, the manometric method was used for measuring the oxygen consumption rate of young flounders. In principle the manometric method involves the movement of a narrow column of fluid in a capillary connected to a flask holding the organism. Respirometers of this type are generally characterised by a U-shaped capillary, or manometer, which measures the change in gas pressure taking place inside the flask. The carbon dioxide, which is released as a product of respiratory activity of the organism, is absorbed. The principle underlying the operation of this class of respirometer is based on the simple gas law equation:

$$PV = nRT$$

Where R is the universal gas constant, P is pressure, V is the volume, n is the number of moles of solute particles and T is temperature in ^{o}Kelvin. This states that if two of the three variables are held constant and a change takes place in the number of molecules of gas in the system, then the consequent change in the third variable is a measure of the change in the amount of gas. The gas equation represents a synthesis of the laws of Boyle, Charles, Gay-Lussac and Avogadro. It has been known for well over a century. It is no surprise, therefore, that

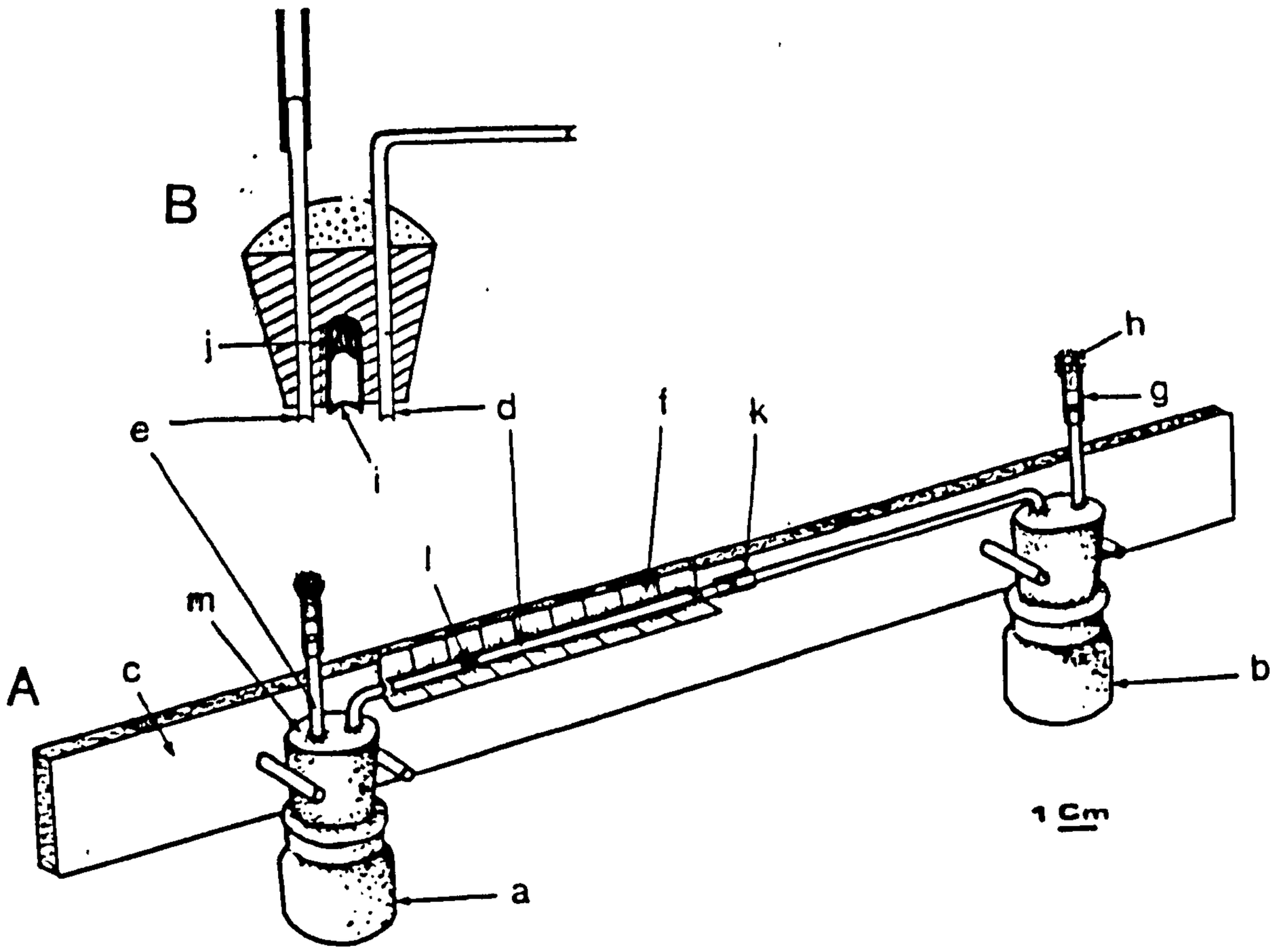
manometric methods are the oldest of all those in current use for the measurements of oxygen consumption (e.g. Barcroft, 1908; Thunberg, 1906).

There are three main types of manometric respirometers. In all three, temperature is held constant as is either the gas pressure or gas volume, or both. In the constant pressure respirometer, the pressure of the gas in the flask holding the organism is maintained at the initial level, whilst the gas volume changes. A constant pressure instrument, which has been in general use for a number of years, is the Gilson respirometer (Winberg, 1956).

Conversely, in constant volume respirometers it is the gas pressure which changes, the gas volume remaining unchanged at the end of the experiment. The Warburg apparatus is a constant volume respirometer, which has been used extensively, particularly in biochemistry. In the differential respirometer, the volume in the flask and the internal pressure change throughout the experiment.

3.2.1 THE RESPIROMETER

Two types of manometric respirometer were used for the determination of oxygen uptake rate in young flounders. The micro-respirometer, which is identical to that of Dixon (1979), is characterised by its simplicity and its sensitivity for use with small animals whose rate of oxygen consumption falls within the range of 0.1-5 $\mu l O_2 h^{-1}$ (Dixon, 1979). For larger individuals, the Gilson respirometer was used. The Dixon respirometer (Fig. 2) consists of two identical vessels ($5cm^3$), one of them being a respiration vessel (2 A, a), and the other a compensation vessel (2 A, b). The vessels are connected by a graduated capillary (2A, d) containing a drop of index fluid (Apeizon oil) (2A, 1). These capillaries are joined by a thin-walled polythene tubing (2A, k). The respirometer is set on a Perspex holder (2A, c) which is supported by a frame (Fig. 3 , b) in a water bath (3 , a). 1mm squared graph paper (2A, f) was lacquered and fixed to the horizontal arm of the capillary tube. No. 17 rubber bungs (2A, m) were drilled to fit a glass well (2B , i) and capillary tubing (2B , e). The wells were made from 10mm lengths of glass tubing, which were sealed at one end and had internal diameter of 5mm (2B , j).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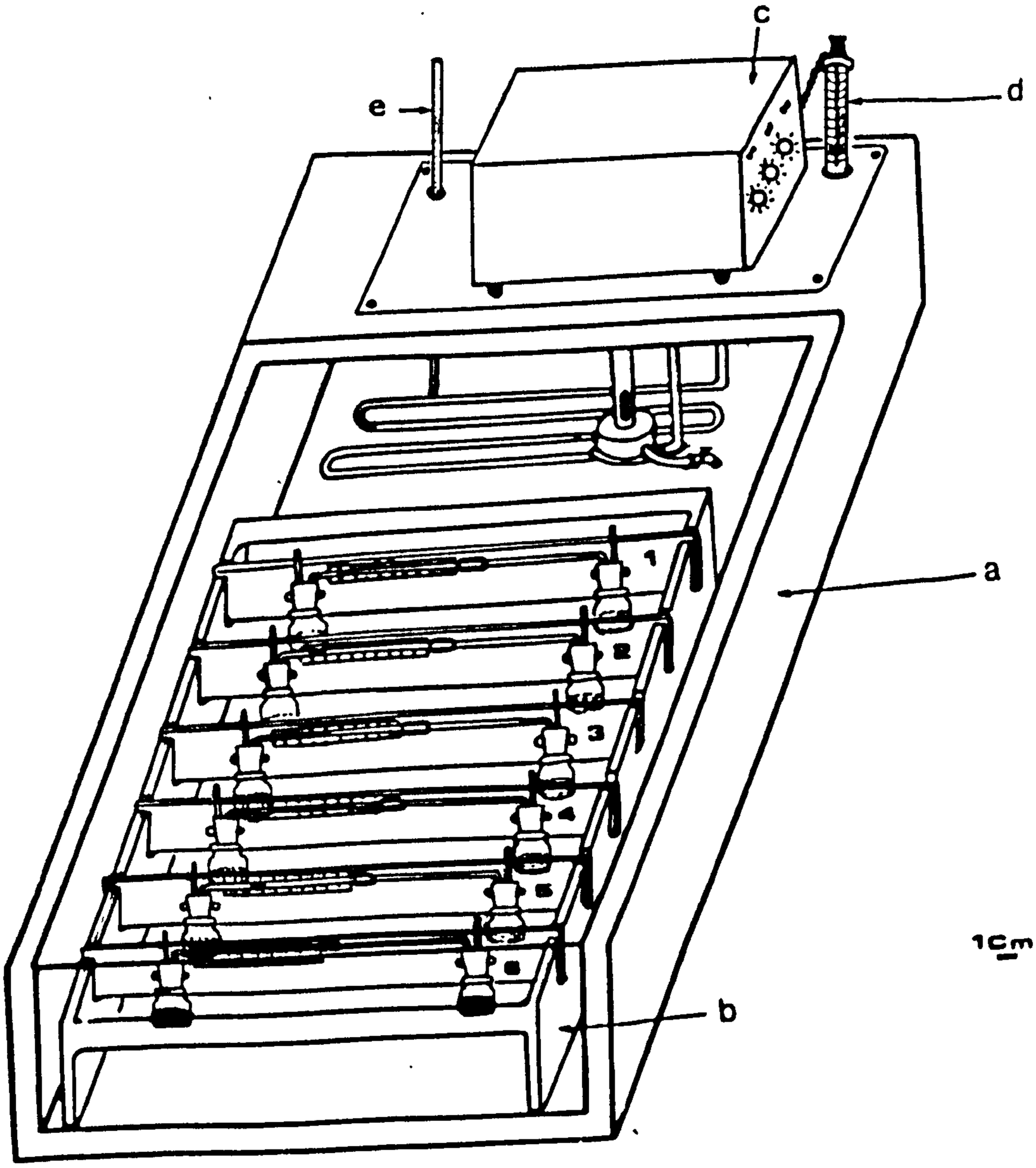
c
e
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1
2
3
1 Cm

The micropipettes (Yankee, Micropet by Clay Adams, USA) were bent into a right angle approximately 30mm from one end using a bunsen flame. Valves were constructed from 40mm lengths of capillary tubing, which were inserted through the rubber bungs, by using 20mm lengths of polythene tubing. A slotted rack, which is shown in Fig. 5, was constructed to accommodate six respirometers. Five of these were experimental and one was a control. These were set approximately 50mm apart in the water bath.

To operate the apparatus successfully, it was necessary that the volumes of gases in the vessels were identical. The internal capacity of the respirometer and compensation vessels (Supplier, F.B.G. Trident Limited, London), when attached to the bungs, was established by using a gravimetric method (Dixon, 1951). The bungs were inserted to the same depth in the vessel every time. The capillary tube was wetted with Apeizon oil to make sure that the index droplet moved freely (Dixon, 1979). A 3mm long drop of Apeizon oil was then inserted into the long arm of the graduated capillary. Once the respirometer was assembled, all the joints were greased with silicon high vacuum grease

(Edwards High Vacuum Limited, Sussex, England).

The respirometer was then tested for leaks by using the following method. The valves were sealed with 3mm of silicon grease. This caused some movement of the index droplet. To prevent the drop from being pushed into the interconnecting polythene tube, the compensation side of the respirometer was closed first. The index droplet was encouraged to move towards the respiration vessel by slightly warming the compensation vessel by hand. The drop returned back towards its original position once the hand was removed. The test was repeated using the respiration vessel. A leak in the respirometer meant that the index fluid did not respond when the vessel was warmed. This was corrected by applying more grease to the joints (Dixon, 1979).

All the vessels, capillary tubing and bungs were kept clean from any trace of bacterial contamination by washing them in sterile water and autoclaving before each experiment. The same quantities of aerated, sterile sea water were placed in the vessels, the quantity being dependent on the size of the fish. To absorb CO_2, filter paper (5mm width, 40mm length) was wetted with 5% KOH and inserted into the glass

well (Fig. 2B,i). The water bath was adjusted until the water-level reached the perspex pegs. The first reading was taken immediately after the system was sealed. A magnifying lens was used for measuring the movement of the index fluid against the scale. The readings in mm of oxygen were converted to μl at S.T.P. by substituting in this equation:

$$X = d\left(2A.\frac{P}{P_o} \cdot \frac{273}{T}\right) \quad \text{(Dixon, 1979)}$$

where,

X = the amount of gas absorbed at S.T.P.,

d = the distance in mm through which the oil moves in the capillary

A = 1.316 mm^2, the cross-sectional area of the capillary

P = the barometric pressure in mmHg at the time the valves were closed

P_o = standard pressure (760 mmHg) and

T = the absolute temperature of the water bath

3.2.2 Q_{10} VALUE

The increase in a rate caused by a 10°C increase in temperature is called the Q_{10}. The Q_{10} values for different temperature ranges were calculated by

using the equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where R_2 and R_1 are the rates of oxygen consumption at two temperatures T_2 and T_1.

3.2.3 STATISTICAL COMPUTATION

The data on oxygen uptake rate for different weight groups of flounder, Platichthys flesus at various temperatures were analysed with respect to body weight by linear logarithmic transformation using the least square regression method. Hewlett Packard 9810A Computer was used for data computation.

3.3 RESULTS

3.3.1 ACCLIMATION PERIOD FOR PLATICHTHYS FLESUS

When an organism is placed in an artificially imposed condition, its internal rhythm is disturbed, which is reflected by its different physiological behaviour. The response from the organism to experimental condition of the laboratory is often termed as acclimation (Hart, 1952; Prosser, 1973; Schmidt-Nielsen, 1979).

When a single flounder, P. flesus was placed in the respirometer, it showed erratic oxygen uptake rate for about 2-3½ hours after which it attained steady levels (Figs. 4 and 5). It was interesting to note that the period of acclimation was shorter (2-2½ hours) for smaller fish (0.009 and 0.015g) than bigger ones (0.03g) which took about 3-3½ hours to adjust to the new experimental conditions (Fig. 5). From Figure 4 it is apparent that the acclimation time required for the same size of fish (0.03g) at different temperatures was shorter at lower temperatures (5, 10°C) than at higher temperatures (15, 20°C).

Because of the erratic respiratory behaviour in the early periods of experimentation, the values for O_2 consumption during the first 3½ hours were not used

FIGURE 4

Graphs showing the erratic and steady oxygen uptake rate for a 0.03g fish before and after acclimation in the respirometer at different (5, 10, 15 and 20°C) temperatures. The last four steady readings were used for the measurements of standard oxygen uptake rate in P. flesus.

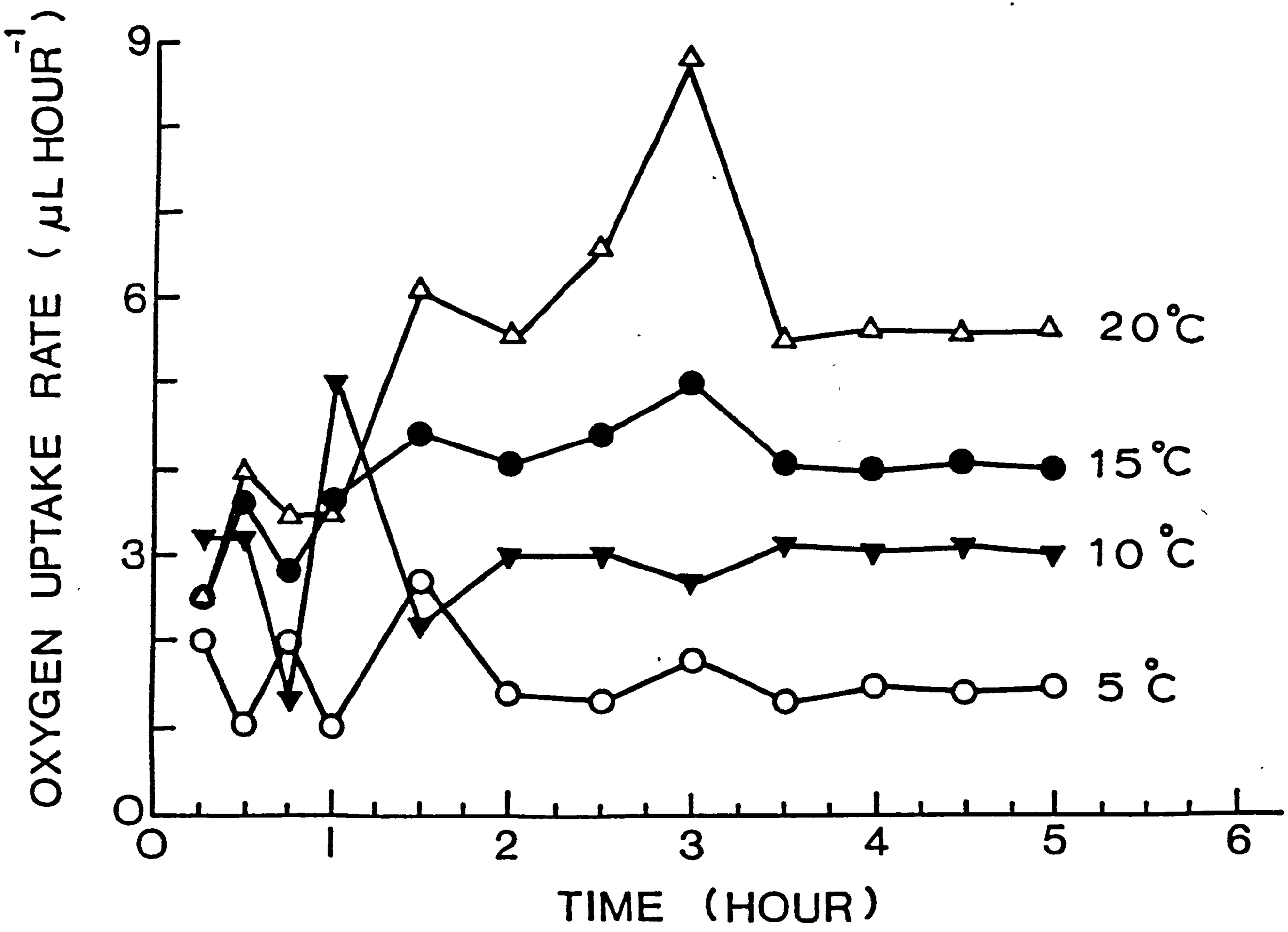
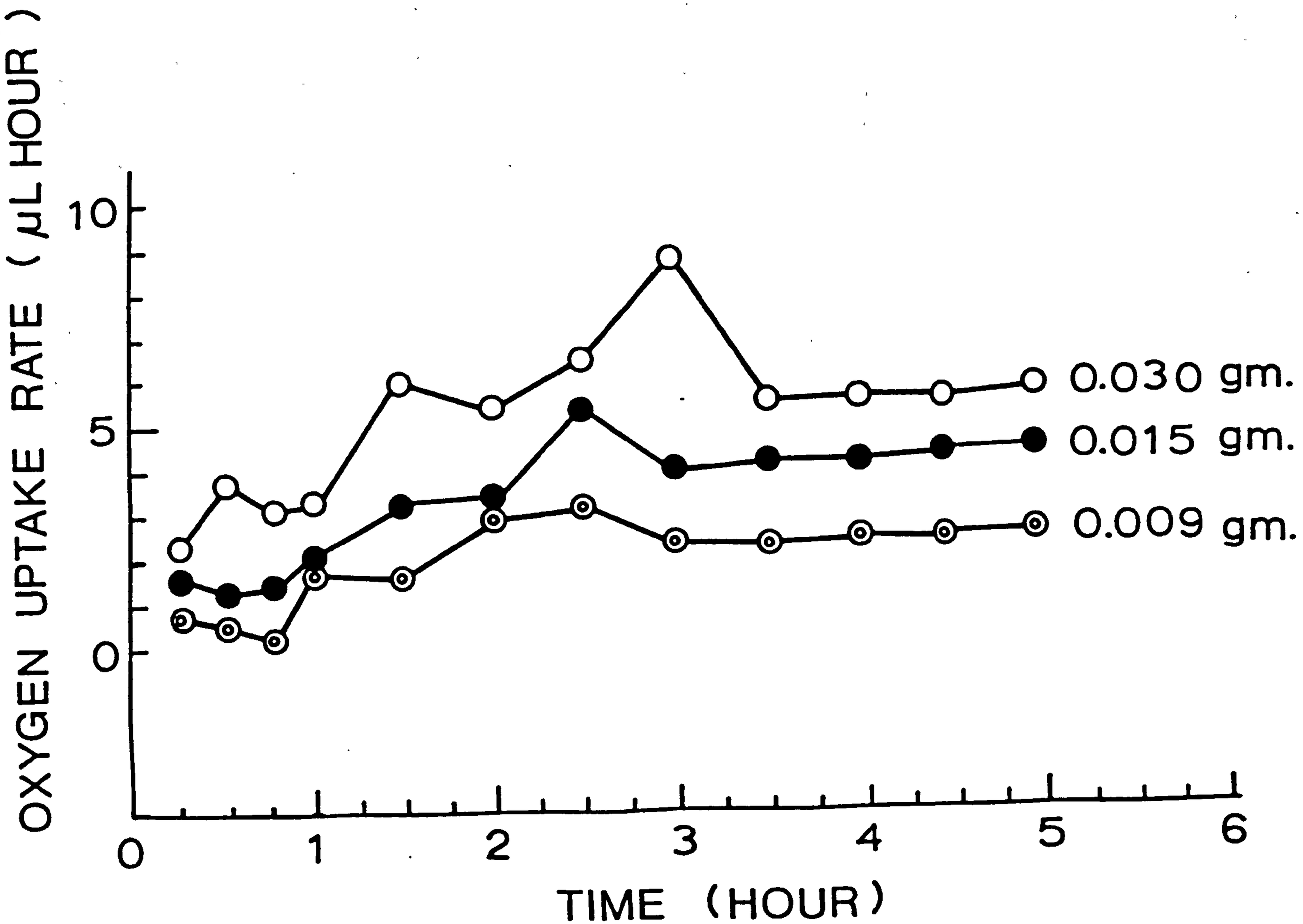

OXYGEN UPTAKE RATE (μL HOUR⁻¹)
9
6
3
0
0
1
2
3
4
5
6
TIME (HOUR)
20℃
15℃
10℃
5℃

FIGURE 5

Simple graphs showing the erratic and steady oxygen uptake rate for a 0.03, 0.015 and 0.009g fish after acclimation in respirometer at 15°C temperature. The last four steady readings were used for the measurements of standard $\dot{V}_{O_2}$ in Platichthys flesus L.


OXYGEN UPTAKE RATE (µL HOUR^{-1})
10
5
0
0
1
2
3
4
5
6
TIME (HOUR)
0.030 gm.
0.015 gm.
0.009 gm.

in the measurement of standard metabolic rate in flounder, Platichthys flesus.

3.3.2 OXYGEN CONSUMPTION RATE IN RELATION TO BODY WEIGHT AND TEMPERATURE

The data for standard oxygen uptake rate for different weight groups of fish at 5, 10, 15 and 20°C are summarised in Tables 1 - 4.

The regression analyses were carried out and the equations showing relationships, body weight and standard O_2 uptake rate at different temperatures are presented in Table 5 and shown in Figures 6-9.

3.3.3 RELATIONSHIP BETWEEN BODY WEIGHT AND $\dot{V}_{O_2}$ STAND. AT 5°C

At 5°C, the standard O_2 uptake rate increased from 2.01 - 11.5 $\mu l O_2 h^{-1}$ in the weight range of 0.009 - 0.045g (Table 1).

When the data for the two variables were plotted on log-log co-ordinates, they gave a straight line with a slope of 1.047 (Fig. 6) and the intercept 0.2188 (Table 5).

FIGURE 6

Bilogarithmic plot showing the relationship between body weight and standard oxygen uptake in P. flesus at 5°C.

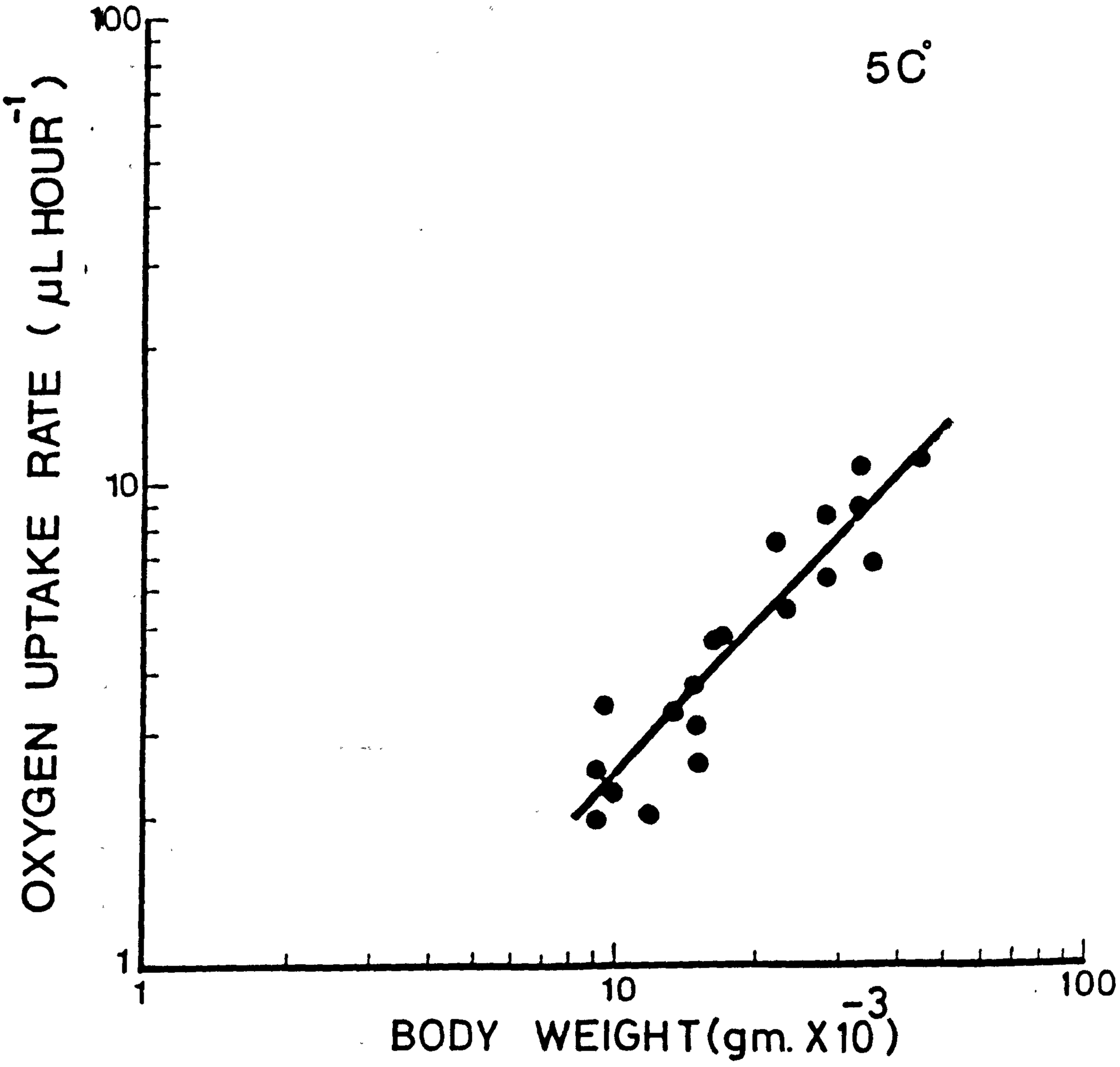

5C°
100
10
1
1
10
100
OXYGEN UPTAKE RATE (μL HOUR⁻¹)
BODY WEIGHT(gm. X 10⁻³)

TABLE 5 : Summary of equations showing relationship between body weight (g) (W), standard oxygen consumption ($\mu l\ O_2 . h^{-1}$) at 5, 10, 15 and 20°C. (Y). Correlation coefficients (r), level of significance (P) and number of specimens are also tabulated.

Temp. (°C)	Equations $Y = aW^b$ or $\log Y = \log a + b . \log W$	Correlation coefficients (r)	level of significance (P)	No. of fish used (N)
5	$Y = 0.2188\ W^{1.047}$ $\text{Log } Y = -0.660 + 1.047 \log W$	0.9630	$P < 0.001$	19
10	$Y = 0.8035\ W^{0.882}$ $\text{Log } Y = -0.095 + 0.882 \log W$	0.8960	$P < 0.001$	25
15	$Y = 1.4555\ W^{0.850}$ $\text{Log } Y = 0.163 + 0.850 \log W$	0.9730	$P < 0.001$	21
20	$Y = 2.0941\ W^{0.801}$ $\text{Log } Y = 0.321 + 0.801 \log W$	0.9900	$P < 0.001$	17

Standard oxygen uptake rate and body weight were highly correlated ($r = 0.963$; $P < 0.001$).

3.3.4 RELATIONSHIP BETWEEN BODY WEIGHT AND $\dot{V}_{O_2}$ STAND. AT 10°C

At higher temperature (10°C), the standard oxygen uptake rate increased from 3.6178 to 34.053 $\mu l O_2 . h^{-1}$ in the weight range of 0.0038 - 0.0609g (Table 2).

When the data on oxygen uptake rate per unit time ($\mu l O_2 . h^{-1}$) were plotted against body weight on log-log co-ordinates, they depicted a straight line with a slope of 0.882 (Fig. 7) and the intercept 0.8035 (Table 5).

The two variables showed high correlation ($r = 0.896$; $P < 0.001$).

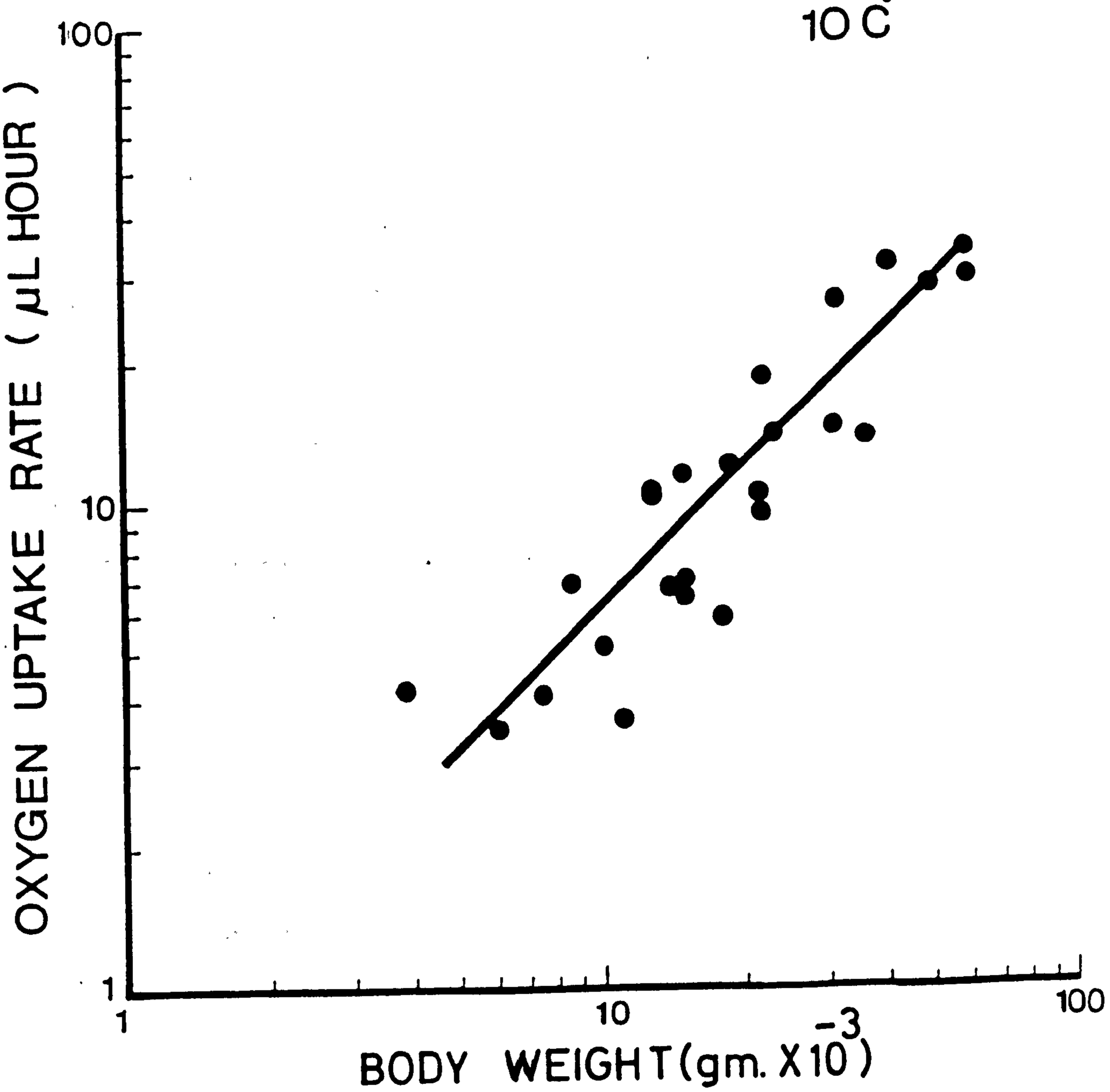
3.3.5 RELATIONSHIP BETWEEN BODY WEIGHT AND $\dot{V}_{O_2}$ STAND. AT 15°C

With the increase to 15°C, the oxygen uptake rate showed higher values. At 15°C, the mean oxygen uptake rate increased from 5.803 to 40.930 ($\mu l O_2 . h^{-1}$) in the weight range of 0.004 - 0.051g (Table 2).

Bilogarithmic plots of $\dot{V}_{O_2}$ rate and body weight gave

FIGURE 7

Bilogarithmic plot showing the relationship between body weight and standard oxygen uptake in P. flesus at 10°C.


10 C°
100
10
1
1
10
100
OXYGEN UPTAKE RATE (μL HOUR -1)
BODY WEIGHT(gm. X 10 -3)

a straight line with a slope of 0.850 (Fig. 8) and the intercept 1.4555 (Table 5).

O_2 uptake rate per unit time and body weight showed high correlation ($r = 0.973$; $P < 0.001$).

3.3.6 RELATIONSHIP BETWEEN BODY WEIGHT AND $\dot{V}_{O_2}$ STAND AT 20°C

Further increased oxygen uptake rate was obtained when the fish were subjected to higher temperature (20°C). At this temperature oxygen uptake rate increased from 11.655 to 60.124 $u10_2.h^{-1}$ in the body weight range of 0.0095 to 0.061 (Table 5).

When the data on O_2 uptake rate ($\mu 10_2.h^{-1}$) was plotted on log-log co-ordinates, they gave a straight line with a slope of 0.800 (Fig. 9) and the intercept 2.0941 (Table 5).

The two variables ($\dot{V}_{O_2}$ and body weight) showed high correlation ($r = 0.990$; $P < 0.001$).

Furthermore, it was interesting to note that lower weight groups (0.001 and 0.01g) of fish showed steady increase of oxygen uptake rate with the rise of temperature from 5 to 20°C. However, in higher

FIGURE 8

Bilogarithmic plot showing the relationship between body weight and standard oxygen uptake in P. flesus at 15°C.

15C°

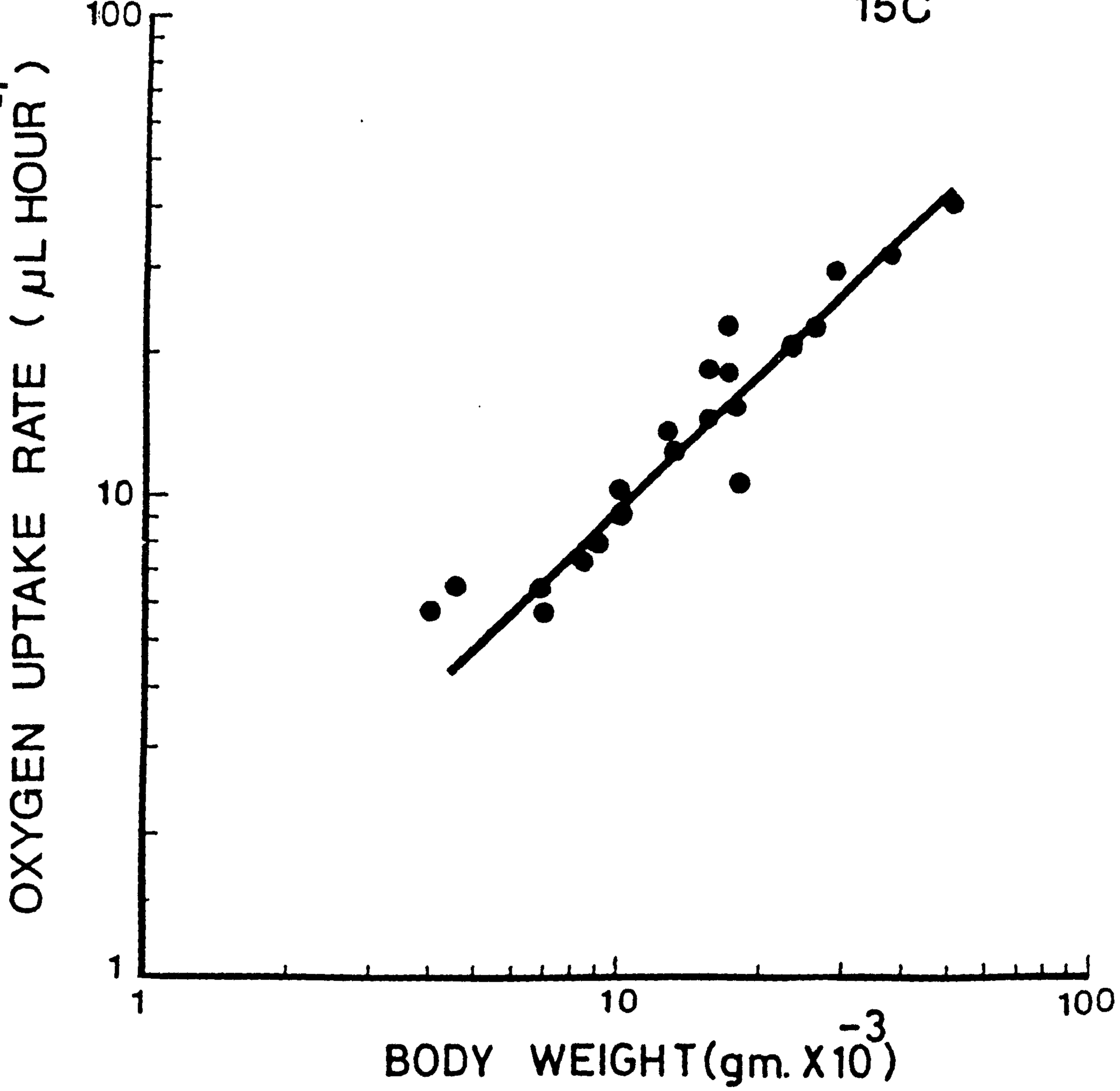
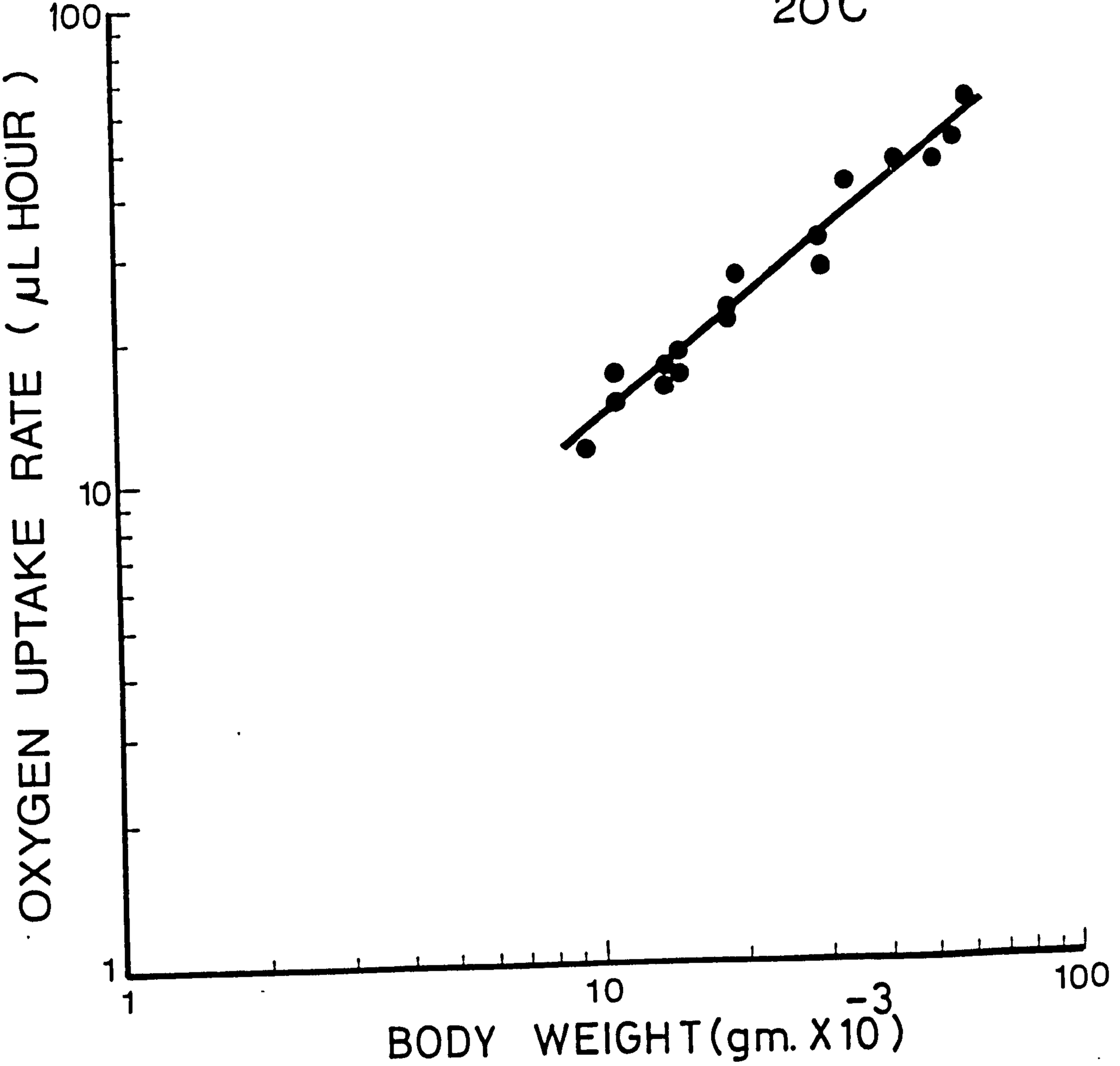

OXYGEN UPTAKE RATE (μL HOUR^{-1})
100
10
1
1
10
100
BODY WEIGHT (gm. X 10^{-3})

FIGURE 9

Bilogarithmic plot showing the relationship between body weight and standard oxygen uptake in P. flesus at 20°C.


20 C°
100
10
1
OXYGEN UPTAKE RATE (μL HOUR -1)
1
10
100
BODY WEIGHT (gm. X 10 -3)

weight groups (0.02, 0.03 and 0.05g), the oxygen uptake rate is steady till 15°C and after that it drops down (Fig. 10).

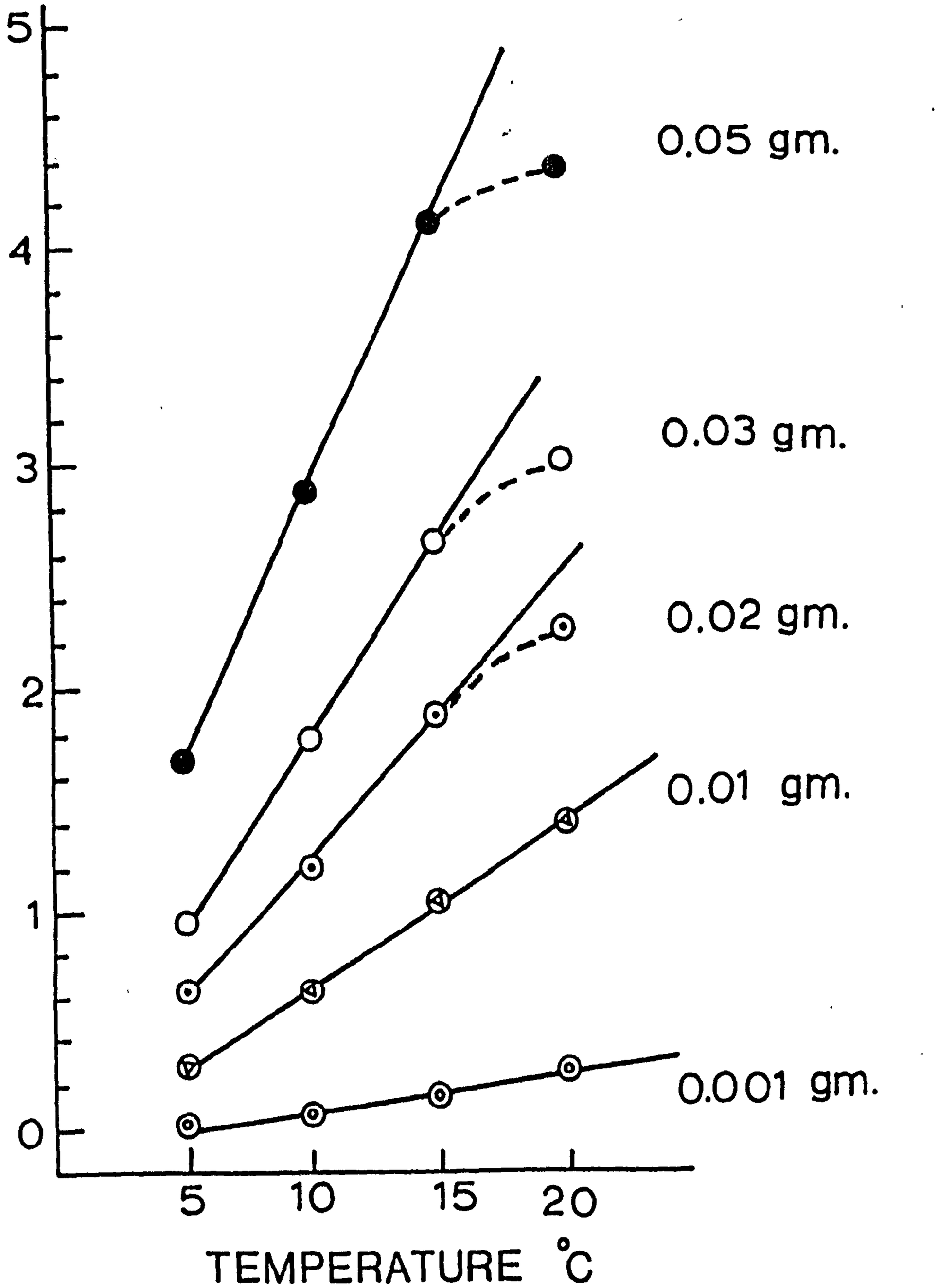
3.3.7 Q_{10} VALUES

Temperature change has striking effects on respiratory physiology of animals. The increase in a rate caused by a 10°C increase in temperature is called the Q_{10}.

In P. flesus, the Q_{10} values were 4.22 and 2.118 at the temperature range of 5 - 15°C and 10 - 20°C respectively.

FIGURE 10

Simple graphs showing a drift in the level of oxygen uptake rate for higher weight groups (0.02, 0.03 and 0.05) of baby Flounders at high temperature (15 and 20°C). The lines for $\dot{V}O_2$ and temperature for small weight ranges (0.001 and 0.01g) remain unaffected.


OXYGEN UPTAKE RATE (µL HOUR⁻¹)
0
1
2
3
4
5
0.05 gm.
0.03 gm.
0.02 gm.
0.01 gm.
0.001 gm.
5
10
15
20
TEMPERATURE °C

3.4 DISCUSSION

Like other organisms, fish require oxygen for aerobic metabolism, which in turn plays a dominant role in generating the energy in the form of adenosine triphosphate (ATP). Oxygen is extracted from the ambient water through various respiratory surfaces. The natural habitat of fishis subjected to adverse ecological conditions, which has a great impact on the total metabolic activities of the animal.

Of the various factors (activity, P_{O_2}, P_{CO_2}, pH, temperature, salinity of ambient water, body dimensions, acclimation and sexual status of the organisms) which influence the oxygen uptake rate (Fry, 1947), the most important factors are body weight, temperature, acclimation and activity.

3.4.1 STANDARD OXYGEN UPTAKE RATE IN RELATION TO BODY WEIGHT

Increase in body mass signifies an increase in living matter, which is associated with higher oxygen uptake rate per unit time. Ever since the relationship between metabolic rate and body weight was discovered much interest has centred on its interpretation and there are few areas of biology that can match the enormous time that has been devoted to

discuss this phenomenon.

Slope (b) of the regression line relating body weight and $\dot{V}_{O_2}$ indicates the trend of oxygen uptake rate per unit time ($ml O_2 . h^{-1}$) with unit increase in body weight. Early estimates considered the exponent value to be about 0.67 which followed the surface area and body weight relationship for organisms of similar geometry. Since the advent of surface law, various exponent values (0.634 - 1.052) have been put forward to explain the relationship between body weight and oxygen uptake rate (Table 6).

Winberg (1960) is of the opinion that a value equal to 0.81 to be an average exponent value for explaining the relationship between oxygen uptake rate and body weight in the majority of fish species. Jager and Dekkers (1975) obtained an average exponent value (b = 0.826) from highly correlated pre-existing data on oxygen uptake rate in relation to body weight (Table 7). They are of the opinion that the oxygen uptake rate and body weight relationship is

TABLE 6 Summarising the slope values (b) for oxygen uptake rate in relation to different fish species at various ambient temperatures.

Fish Species	Temp. (°C)	Weight range	slope (b-values)	References
Platichthys flesus	5	0.009 - 0.045	1.047	Present study
" "	10	0.0038 - 0.0600	0.889	
" "	15	0.004 - 0.051	0.840	
" "	20	0.0095 - 0.0560	0.801	
Platichthys stellatus	14-15	10 - 250	0.842	Hickman (1959)
Parophrys vetulus	14-15	4 - 200	0.842	
Citharichthys stigmaeus	14-15	4 - 40	0.905	
Pleuronectes platessa	10	0.015 - 10	0.721	Edwards et al (1969)
Limanda limanda	10	0.1 - 10	0.666	
Pseudopleuronectes americanus	20	4 - 50	0.790	Voyer & Morrison (1971)
	10	4 - 50	0.730	
Synoglossus spp	28	1 - 200	0.734	Edwards (1971)
Brachurus synoptera	28	1 - 100	0.682	
Salvelinus fontinalis	10		0.849	Job (1955)
" "	15		0.847	
" "	20		0.802	
Salmo trutta			0.877	Beamish (1964)
Salvelinus fontinalis			1.052	
Catostomus commersoni			0.864	
Letalurus nebulosus			0.925	
Cyprinus carpio			0.894	
Cyprinus carpio	20		0.76	Scholander et al (1953)
Small carp			0.98	Winberg & Khartova (1953)
Pleuronectidae (family)			0.71	Zeuthen (1947)
Microstomus kitt (lemon	5	5 - 300	0.783	Duthie (1980)
" " sole)	10	5 - 300	0.717	
" "	15	5 - 300	0.680	
Limanda limanda (dabs)	5	1 - 300	0.772	
" "	10	50 - 300	0.782	
" "	15	5 - 300	0.634	

TABLE 7 Table summarising the relationship between body weight and oxygen uptake rate in various fish species (from Jager and Dekkers, 1975).

Fish Species	$\dot{V}_{O_2}$ Equations	References
Acipenser stellatus	Log $\dot{V}_{O_2}$ = 0.735 log W - 0.239	Clausen, 1936
Anguilla anguilla	Log $\dot{V}_{O_2}$ = 0.794 log W - 0.558	Byczkowska-Smyk, 1958
Catostomus commersonii	Log $\dot{V}_{O_2}$ = 0.864 log W - 0.666	Saunders, 1962
Cyprinus carpio	Log $\dot{V}_{O_2}$ = 0.856 log W - 0.730	Saunders, 1962
Ictalurus nebulosus	Log $\dot{V}_{O_2}$ = 0.925 log W - 1.077	Saunders, 1962
Pleuronectes platessa	Log $\dot{V}_{O_2}$ = 0.786 log W - 0.358	Hughes, 1966
Pseudopleuronectes americanus	Log $\dot{V}_{O_2}$ = 0.756 log W - 0.629	Voyer & Morrison, 1971
Salmo gairdneri	Log $\dot{V}_{O_2}$ = 0.864 log W - 0.433	Hughes, 1970
Salmo trutta	Log $\dot{V}_{O_2}$ = 0.877 log W - 0.446	Hughes, 1966
Tinca tinca	Log $\dot{V}_{O_2}$ = 0.798 log W - 0.483	Hughes, 1970b
Mean regression line	Log $\dot{V}_{O_2}$ = 0.826 log W - 0.562	

best explained by the exponent value equal to 0.82.

The average exponent value (0.82) put forward by Jager and Dekkers is arbitrary in the sense that it is an average of the values obtained from 10 selected values (b) and that (0.81) reported by Winberg (1960).

The exponent values for the two parameters do not only differ in different species but also in the same species under different experimental conditions.

The slopes of the regression lines relating oxygen rate per unit time and body weight in 0-group (0.001 - 0.01g) of Platichthys flesus at four (5, 10, 15 and 20°C) temperatures are all higher (Table 8) than the average of those values reported by Winberg (0.81) and Jager and Dekkers (0.82). Higher exponent values in small weight range of P. flesus may be due to their higher metabolic rate. 10°C ambient temperature seems to be the optimum temperature for the metabolic activities in these fish as they experience the same thermal condition in their natural habitat. Therefore, a value equal to 0.89 may be a more representative coefficient for the 0-group of P. flesus.

3.4.2 STANDARD OXYGEN UPTAKE RATE IN RELATION TO TEMPERATURE

The temperature of an animal's body generally has a profound effect on its metabolic functions. The various cellular components of the organism have upper and lower lethal temperatures and within this thermal range, there is an optimum temperature limit

TABLE 8. Summary of the oxygen uptake rate ($u1O_2/h$) at 5, 10, 15 and 20°C for specimens of 0.001, 0.01, 0.1, 1, 10 and 100g body weight, together with 95% confidence limits computed from regression lines using logarithmic transformations. Standard deviations for the slope (Sb) and the intercept (Sa) of the regression lines and the number of specimens (N) used for the computation are also given.

Temp. (°C)	0.001 g	95% CL	0.01 g	95% CL	0.1 g	95% CL	1g	95% CL	10g	95% CL	100g	95% CL	Sb	Sa	N	b-value
5	0.2192	0.4129 0.1163	2.4422	2.8836 2.0684	27.2141	39.9030 18.5602	303.2534	724.2493 126.9765	3379.2242	1.3225×10^4 863.4264	$2.765^6 \times 10^4$	2.4181×10^5 5863.9285	0.1021	1.5107	19	1.0470
10	0.8079	1.4173 0.4606	6.2548	7.4132 5.2775	48.4234	69.0855 33.9409	374.8835	817.6549 171.8789	2902.2658	9774.1563 861.7774	2.2469×10^4	1.1709×10^5 4311.7032	0.0920	1.4578	25	0.8888
15	1.4560	2.1128 1.0034	10.2994	11.3639 9.3347	72.8548	97.3030 54.5494	515.3517	939.6577 282.6426	3645.4359	9120.0227 1457.1458	$2.5787^4 \times 10^4$	8.8620×10^4 7503.4496	0.0661	1.3324	21	0.8497
20	2.0933	2.7289 1.6057	13.2213	14.3738 12.1613	83.5071	95.5837 72.9562	527.4381	728.2676 381.9901	3331.3465	5571.736 1991.8153	2.1041×10^4	4.2661×10^4 1.0378×10^4	0.0394	1.1635	17	0.8005

at which the enzymes carry out different metabolic functions efficiently. Out of the several different types of relationship which animals display with their thermal environment, the two particularly prevalent types of thermal relationships are homeothermy (endothermy) and poikilothermy (ectothermy). Fish experience the latter type.

As in all poikilothermic organisms, the oxygen uptake rate of fish is determined by equilibration with the thermal conditions of the environment. Krogh (1916) attempted to ascribe a general equation relating metabolism and temperature. With the help of his experimental data on organisms of very diverse systematic positions, it was concluded that the 'standard metabolism' of an organism is subjected to a general quantitative law common to all groups, which can be expressed by a curve known as Krogh's normal curve.

Since then 'Krogh' normal curve has gained great popularity and was confirmed by various experimental data. 'Krogh's normal curve' received support from the experimental data of Lindstedt (1914) on tench, Gardner (1926) on different fish species, Keys (1931) and Wells (1935) on Fundulus parvipinnis, Stroganov

(1939) on Gambusia holbrook, Svirenko (1937, 1948), Scholander et al (1953) on four tropical and arctic fish over the thermal ranges of 15 - 35°C and 0 - 15°C respectively.

Although 'Krogh's normal curve' describes the effect of temperature on metabolism, the relationship between the two parameters is empirical and cannot be expressed by a simple equation. For this reason the thermal sensitivity of metabolic rate is expressed by the temperature coefficient, Q_{10}. It is defined as the ratio of the oxygen uptake at the two adaptational temperatures, i.e.

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where R_2 and R_1 are oxygen uptake values at temperatures T_1 and T_2.

Like other poikilotherms, the 0-group flounders are also very sensitive to the thermal status of the ambient water. The oxygen uptake rate ($\dot{V}_{O_2}$) is accelerated with an increase in the ambient temperature (Table 6). An increase in oxygen uptake rate with increase in temperature is obviously due to a greater metabolic requirement at higher temperatures.

Different Q_{10} values (6.58, 4.22, 2.71 and 1.65) for P. flesus at various temperature ranges (5 - 10, 5 - 15, 10 - 15 and 15 - 20°C) respectively indicate relative sensitivity of metabolism of the fish to various temperature ranges.

Metabolism of 0-group flounders is very sensitive between 5 - 15°C. The higher Q_{10} values for P. flesus between 10 - 15°C and 10 - 20°C indicate substantial reduction in metabolic processes at low temperature. The activity of fish decreases with the fall of ambient temperature and this may be one of the reasons for higher Q_{10} value between initial temperature range. Reduction in metabolism at low temperature may be an advantage for fish as they may conserve metabolic reserve food materials in the winter and utilize them for increased metabolic rate during favourable environmental conditions. Higher Q_{10} values for lower thermal ranges have also been reported by Timothy (1978) in Fundulus heteroclitus.

Rise in temperature also affects the slope of the regression line relating $\dot{V}O_2$ and body weight in flounders (Table 8). At higher temperatures, comparatively lower slope values indicate greater decrease in weight specific oxygen uptake rate.

Higher weight groups of fish show a decrease in oxygen uptake rate at higher temperature (15/20°C). This decrease in oxygen uptake rate may have a depressing effect on the exponent values at higher temperature.

3.4.3 OXYGEN UPTAKE RATE IN RELATION TO ACCLIMATION

All organisms respond to artificially imposed experimental conditions in the laboratory, which in turn affect their metabolic activities. For this reason, experimental animals are acclimated to various laboratory conditions. 0-group of P. flesus were acclimated to various temperature ranges for about one month before they were tested for metabolic rate at different acclimation temperatures. Even then, in the respirometers, these fish showed erratic respiratory behaviour before settling down for stable metabolic activities. In these experimental fish, the acclimation period seems to be size dependent as smaller fish (0.009 - 0.015g) take a shorter period for acclimatisation in comparison to larger weight (0.03g) group of fish.

C H A P T E R 4

ORGANIZATION OF THE GILLS OF FLOUNDER, PLATICHTHYS FLESUS (L.)

Chapter 4: ORGANIZATION OF THE GILLS OF FLOUNDER, PLATICHTHYS FLESUS (L)

4.1. INTRODUCTION

Fish gills are designed to extract oxygen efficiently from water, which contains about 1/30th of oxygen present in the same volume of air. The fish gills are such a fascinating subject for fish biologists that some have introduced multidisciplinary investigations in attempts to understand their structure and function.

The gross and microscopic structure of fish gills have been studied by a number of workers in the past. Among them Duvernoy (1839), Reiss (1881), Bietrix (1895), Plehn (1901), Faussek (1902), Schottle (1932), Rauther (1937) and Bijtel (1947, 1949) deserve special mention.

Duvernoy (1839) gave his main attention to the muscles concerned in the movement of the gill filaments. He distinguished two types of muscles and called them adductors. Reiss (1881) studied the microanatomy of the gill filaments of Esox, Perca fluviatilis, Salmo salar and some of Cyprinoidea. He distinguished two types of gill filament muscles - the abductors and the adductors. Bijtel (1947, 1949) studied the structure of the gills of twelve species of teleosts, including Esox lucius, Tinca tinca, Cyprinus carpio and Salmo

irideus. She dealt especially with the gross anatomy of the gill filaments and the muscles concerned in their movements and shown that "the wide apertures between the gill are closed in quiet respiration during inspiration as well as expiration. The adduction movements of the filaments, however, are seen only in connection with the 'coughing' movements". Munshi (1960) made a similar distinction between two types of filament adductor muscles depending on their position along the filament. The filament abductor and adductor muscles of teleosts seem to be homologous with the constrictores branchiales of the Selachians. It is quite likely that gill rays of one holobranch, originally arranged in a single row on the gill arch as in the elasmobranchs have shifted slightly in the position in such a way as to make the gill lamellae of a holobranch alternate or interdigitate. This shift of the gill rays must have affected the position of the fasciculi of the deep constrictor muscles which were attached with them. The most proximal part of the deep constrictor muscles (Pars branchialis) retains its original position between the gill arch and the gill rays in the form of the abductor muscles. This explains the position of the abductor sheet on the anterior or outer side of the gill arch and its absence on the other side. The pars inscriptionalis is broken up into several muscles forming the filament adductors of teleosts which lie inbetween the gill rays (Munshi, 1960; Hughes & Morgan, 1973). These small muscles have

an important role during gill ventilation as their activities produce expansion and contraction of the gill curtain in relation to the changing thrust of buccal pressure and opercular suction pumps (Munshi, 1967). Pasztor and Kleerekoper (1962) gave detailed accounts of the functional organization of gill filaments. Munshi, Ojha and Mittal (1975) and Ojha and Munshi (1976) recognised different types of muscle fibres in abductor and adductor muscles of the constrictor branchialis by their succinic dehydrogenase (SDH) activities. They observed an intense reaction in the adductor muscles which consist entirely of red fibres and are therefore metabolically more active. By its weak SDH reaction, the abductor muscle of gill filaments was considered to have low level of metabolic activities. These histochemical activities support the previous physiological findings of Pasztor and Kleerekoper (1962) that adductor muscle units showed slow and long-lasting contraction rhythms that the abductor muscles are used during coughing.

Bietrix (1895), Plehn (1901) and Faussek (1902), Scribran (1931) and Acrivo (1938) were concerned with the discovery of pillar cells in the secondary lamellae of the fish gills. Bietrix (1895), Hughes and Grimstone (1965), Newstead (1967), Hughes and Weibel (1972), Hughes and Wright (1970) and Hughes and Munshi (1973a,b) described in detail the structure of pillar cells and

their relationship with basement membrane and columns.

The first studies of pillar cells using electron microscopy (Hughes and Grimstone, 1965; Newstead, 1967) revealed the presence of well defined filaments arranged in parallel bundles. Furthermore evidence for a myosin-like contractile mechanism obtained by Bettex-Galland and Hughes (1973) has been confirmed using immuno-fluorescence histochemistry (Smith and Chamley-Campbell, 1981). Such a mechanism suggested a possibility of controlling the blood flow through the secondary lamellae (Hughes and Grimstone, 1965).

The histochemical nature of the basement membrane and columns has been described by Scriban (1931), Munshi (1960) and Munshi and Singh (1968).

Rauther (1937), Acrivo (1938), Hughes and Grimstone (1965), Hughes (1970), Muir (1970), Tovell, Morgan and Hughes (1970), Hughes and Morgan (1973) and Muir and Brown (1971) studied the distribution of pillar cells in the secondary lamellae.

In addition to the aforesaid structural units, the gills also contain many branchial glands which are concerned with ionic regulation between blood and the ambient water. Functions of the branchial glands have been studied in the past by Keys and Willmer

(1932), Bevelander (1936), Copeland (1948), Burns and Copeland (1950), Vickers (1961), Philpott (1965), Shirai and Utida (1970), Ojha and Munshi (1974) and others.

Fish gills also provide an interesting architectural plan of the respiratory and haemodynamic systems. The respiratory (arterio-arterial) and nutritive (arterio-venous) streams are the two pathways by which blood circulates through the various components of teleostean gills. The respiratory path of the blood flow through the gills has been well established (Allis, 1912; Goodrich, 1930; Mott, 1950, 1951; Muller, 1839; Munshi and Singh, 1968; Muir, 1970). However, little is known of the non-respiratory (arterio-venous) pathway of blood in fish gills (Reiss, 1881; Steen and Kruysse, 1964; Richards and Fromm, 1969; Vogel et al., 1973, 1974, 1976; Laurent and Dunel, 1976).

The present chapter of the thesis is an attempt to elucidate the functional organisation of the gills of the flounder, Platichthys flesus.

4.2 MATERIALS AND METHODS

Specimens (1-50g) of Platichthys flesus were obtained from the Plymouth laboratories of the Marine Biological Association (U.K.). The fish were anaesthetised in MS222 (0.01 $g.l^{-1}$) and a few gill filaments were fixed at 0-4°C for one hour in 5% glutaraldehyde with a few drops of collidine and washed and post-fixed for one hour in 1% osmium tetroxide; both were buffered at pH 7.4 with Marine Teleost Saline (Young, 1933).

Fixed materials were embedded in araldite and thin 1 µm transverse and sagittal serial sections of gill filament were obtained using an LKB Ultratome III, and stained in 1% Toluidine blue and 1% Azur in 1% Borax (Richardson, Jarret and Finke, 1960).

Ultrathin sections were also cut with LKB Ultratome III, stained with uranyl acetate (Waston, 1958) and lead nitrate (Venable and Coggeshall, 1965) and viewed in Phillips 300 and EM 6G electron microscope.

4.3 RESULTS

Gills of Platichthys flesus show a similar anatomical configuration to that found in other teleostean fish. Four pairs of gills are present in the asymmetrical opercular cavities (Fig. 27). Each gill arch bearing two rows of gill filaments comprising the two hemi-branchs, anterior and posterior. The gill filaments of each hemibranch lie parallel to each other and perpendicular to the surface of the arch. An alternating series of flattened secondary lamellae are arranged in two rows from the sides of the filaments (Plate 2).

The water/blood barrier in the secondary lamellae consists of three layers:

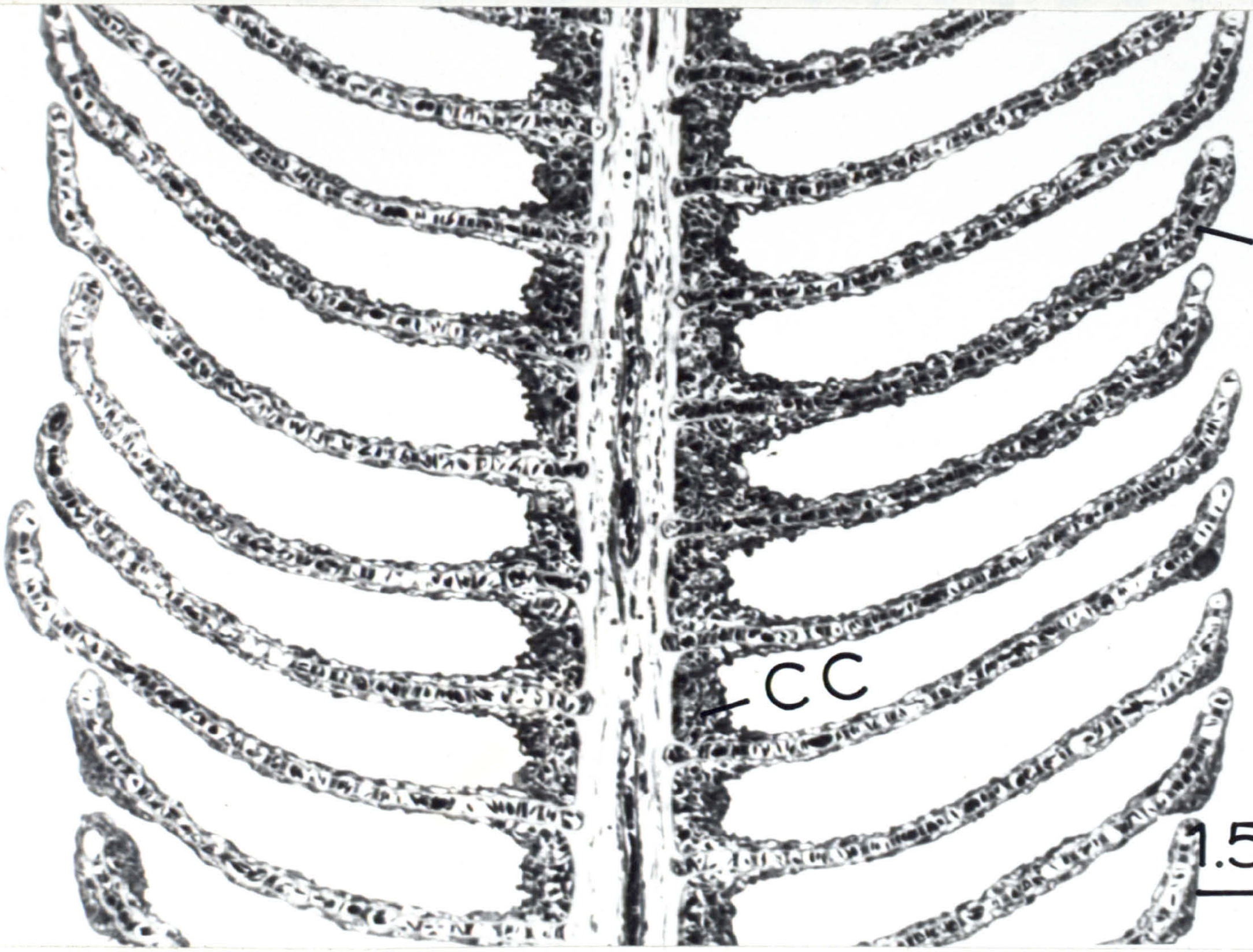
1. The outer layer of epithelial cells, which consists of one or more layers of cells,
2. The middle layer, which is called the basement membrane, consists of three layers - an outer, clear layer; a middle, fine fibrous layer (the basal lamina); and an innermost is the collagenous layer, and
3. The inner flange of pillar cells (Plate 5a).

In most instances the nuclei of epithelial cells are located immediately over the pillar cells (Plate 3a). Each epithelial cell at its outer surface shows the presence of small microridges covered with a thin

PLATE 2

Light micrograph of a longitudinal section of a gill filament showing the alternating arrangement of secondary lamellae (SL). Note the parts of the lamellae embedded in the filament epithlia which consist mainly of chloride cells(CC).

(X 150; X 100)


SL
CC
1.5mm.


1mm.
CC

mucus layer (plate 6c).

4.3.1 PILLAR CELLS

The pillar cells are roughly cylindrical in shape and separate the two layers of basement membrane of a secondary lamella. The ends of each pillar cell extend to give off the flange of pillar cells. These flanges of pillar cells run immediately below the basement membrane and line the network of blood channels of the secondary lamellae (Plates 3a, 4). The flanges of adjacent pillar cells meet and inter-connect closely and form the boundary of the blood spaces and isolate them from the tissues of the other layers of the water/blood barrier. Overlapping of adjacent pillar cell flanges has been noticed at the point where they meet (Plate 3b). However, at the outer edge of the secondary lamellae, pillar cell flanges only line the proximal part of the marginal blood channels (Plates 3a, 4).

Each pillar cell has a large centrally placed nucleus (Plate 4). Four to eight columns are discernible in association with each pillar cell (Plate 5b). Pillar cell columns composed of collagen fibres are continuous with the connective tissue layer of the basement membrane on both sides of secondary lamella. The columns are extracellular structures which remain enveloped by intucking of plasma membrane of pillar cells.

PLATE 3

a. Electron micrograph of section through secondary lamellae showing the marginal channel (MCH). The nucleus of an endothelial cell (EN) is clearly visible. The epithelial layers (EP), basement membrane (BM), and pillar cell flange (PCF) complete the water/blood barrier. Note the junction between the endothelial cells and a pillar cell flanges. A red blood corpuscle (BC) are seen in the blood space (BS). Nucleus (N), pillar cell (PC), endoplasmic reticulum (EN) are also shown.

(X 11,500)

b. Higher magnification electron micrograph of a part of the junction of a pillar cell flange (PCF). Note the desmosome (D) between epithelial cells (EP) of filament epithelial layer. Nucleus (N), collagen (COL), basement membrane (BM), and pillar cell (PC) are also shown.

(X 64,000)

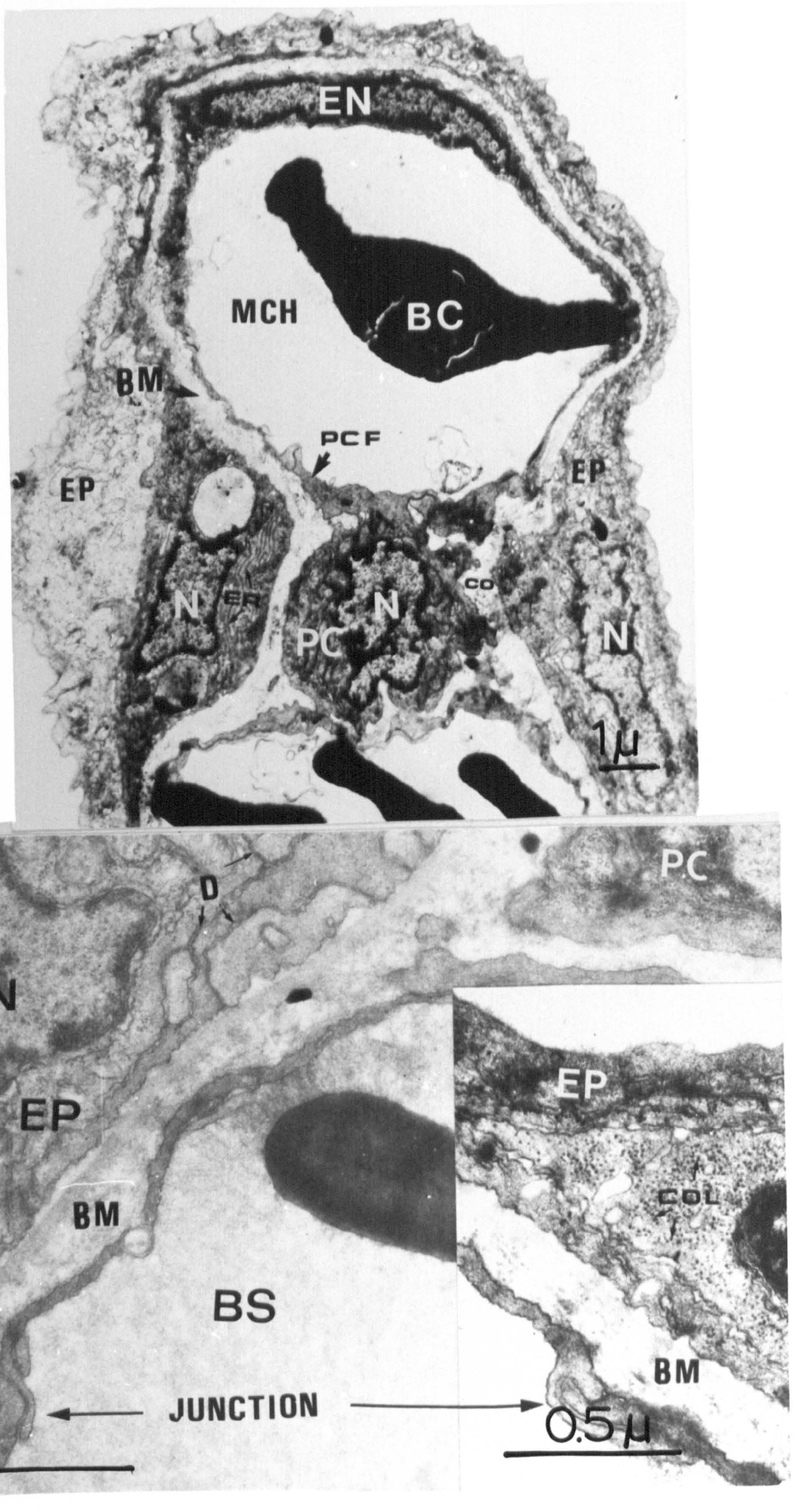
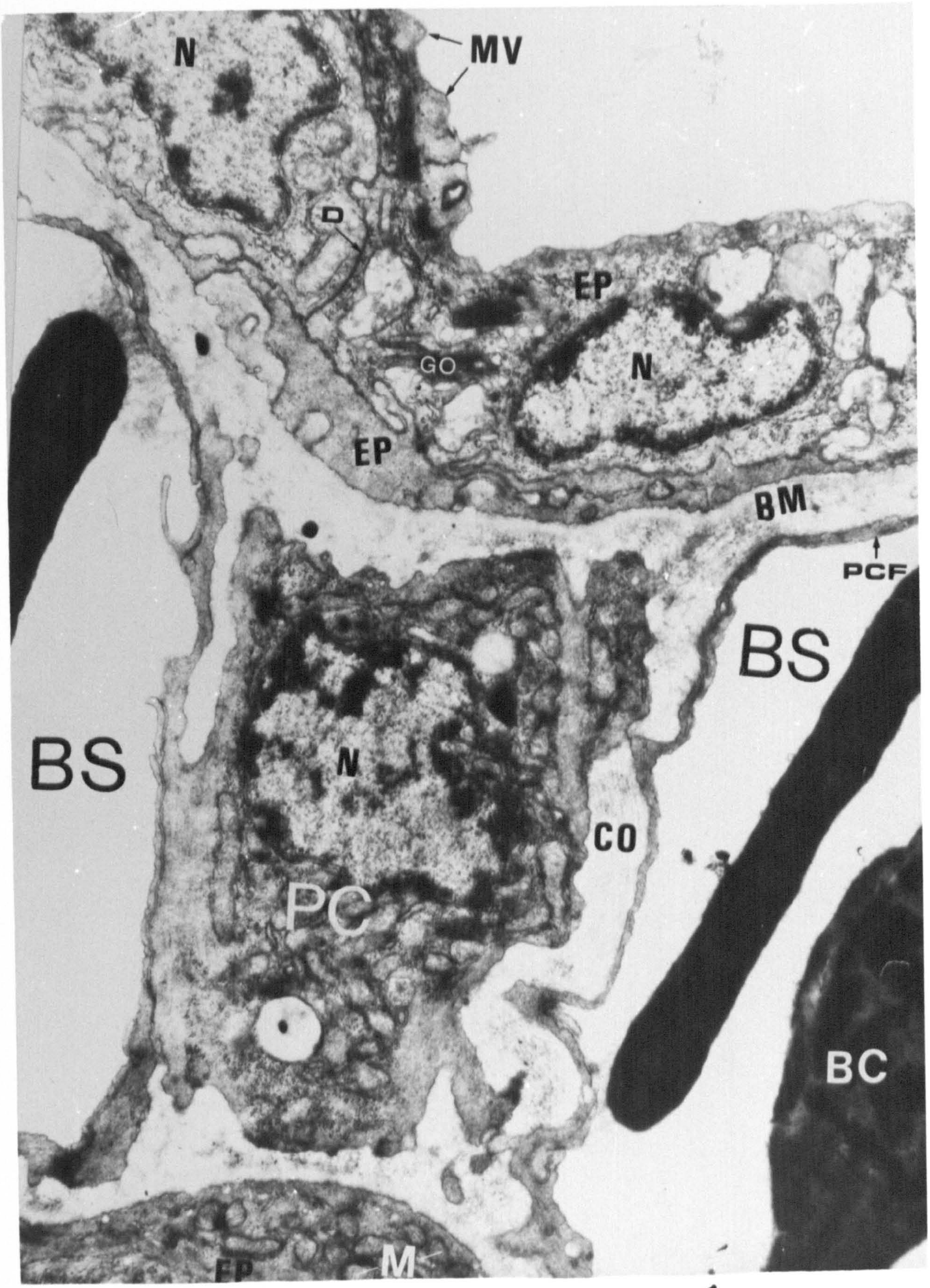

EN
MCH
BC
BM
PCF
EP
EP
N
ER
N
PC
N
CO
N
1μ
P.C
D
N
EP
EP
COL
BM
BS
JUNCTION
BM
0.5μ

PLATE 4

Electron micrograph of section through secondary lamellae showing a pillar cell body (PC) and its flanges (PCF) separating two of the blood channels space (BS). Note the large nucleus (N), and the abundant mitochondria (M). A column (CO) is present, enclosed in an infolding of plasma membrane. In the epithelia (EP) note the location of the nuclei opposite the pillar cell body and the presence of more than one cell layer. Golgi apparatus (GO), basement membrane (BM), desmosome (D), microvillus (MV), lamellar body (LB), are also shown.

(X 48,000)

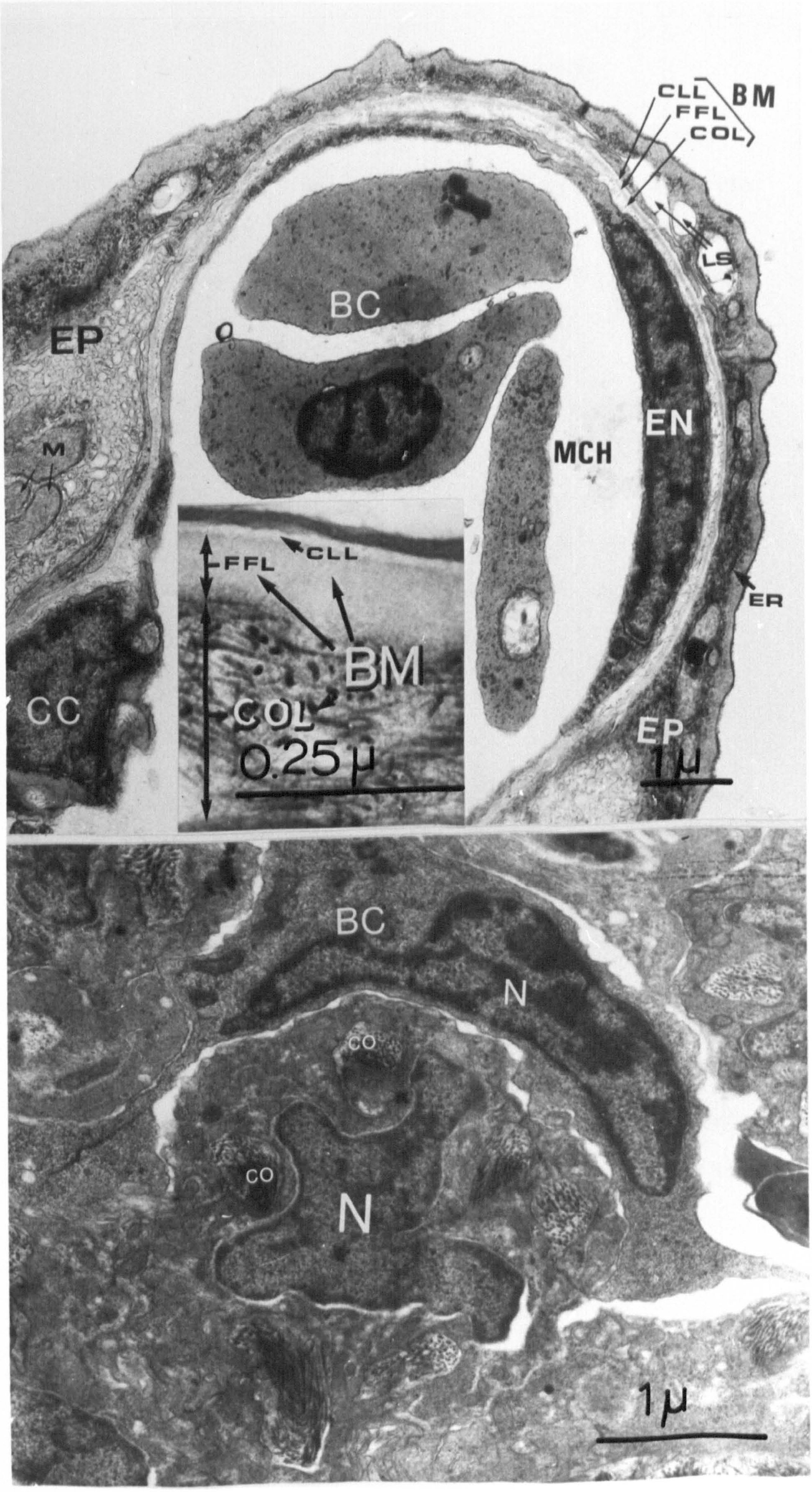


1μ

PLATE 5

a. Electron micrograph of section through secondary lamella showing the marginal channel (MCH) of the secondary lamella lined with endothelial cells (EN), which extend from the distal pillar cell flange (PCF). Note the lymphoid space (LS) beween epithelial layers (EP). Also, higher magnification electron micrograph of a part of the basement membrane (BM), showing the three layers an outer, clear layer (CLL); a middle, fine fibrous (FFL); and an innermost is the collagenous layer (COL). Marginal channel (MCH), mitochondria (M), are also shown.
(X 17,000; X 165,000)

b. Electron micrograph of section in the plane of a secondary lamella showing pillar cells cut transversely with their large nucleus (N), also 6 columns (CO) , abundant mitochondria, and some of the blood cells (BC) are shown.
(X 32,000)


CLL
FFL
COL
BM
LS
BC
EP
M
CC
FFL
CLL
BM
COL
0.25 μ
MCH
EN
ER
EP
1μ
BC
N
CO
CO
N
1μ

4.3.2 BLOOD SPACES AND BLOOD CORPUSCLES

From the aforesaid account, it is evident that the blood channels of the secondary lamellae are entirely delimited by the pillar cells and their flanges. However, marginal channels of the secondary lamellae are partly lined by endothelial cells (Plates 3a, 5a).

The erythrocytes are nucleated with well-defined nuclear envelopes. Occasionally mitochondria have been observed in close association with the nucleus of the erythrocytes. Marginal channels have greater numbers of erythrocytes than other blood channels of the secondary lamellae (Plate 5a).

4.3.3 BRANCHIAL GLANDS

In P. flesus mucous and mitochondria-rich cells have been found associated with gills.

The mucous cells are present on the gill bar region and primary epithelium of the filaments (Plate 6a, b). The mitochondria-rich cells are present in the primary epithelium of the filaments between secondary lamellae. These cells contain numerous mitochondria (Plate 8b).

Each mitochondria-rich cell is very closely associated on the one hand with blood channels of secondary lamellae and on the other hand opens through a pit on the surface of the epithelium (Plate 7b). About 2-3 blood channels of the secondary lamellae make close

PLATE 6

a. Electron micrograph of mucous cell showing apical aperture surrounded by overlying extension of epithelial cells (EP). Note the mucus droplets (MUD) inside the mucous cell.

(X 26,000)

b. Electron micrograph of mucous cell in the epithelial cells (EP). Note the large nucleus (N).

(X 21,000)

c. Electron micrograph showing the mucous layer overlying the epithelial cells (EP). Basement membrane (BM), golgi apparatus (GO), lymphoid space (LS), blood space (BS) are also shown

(X 32,000)

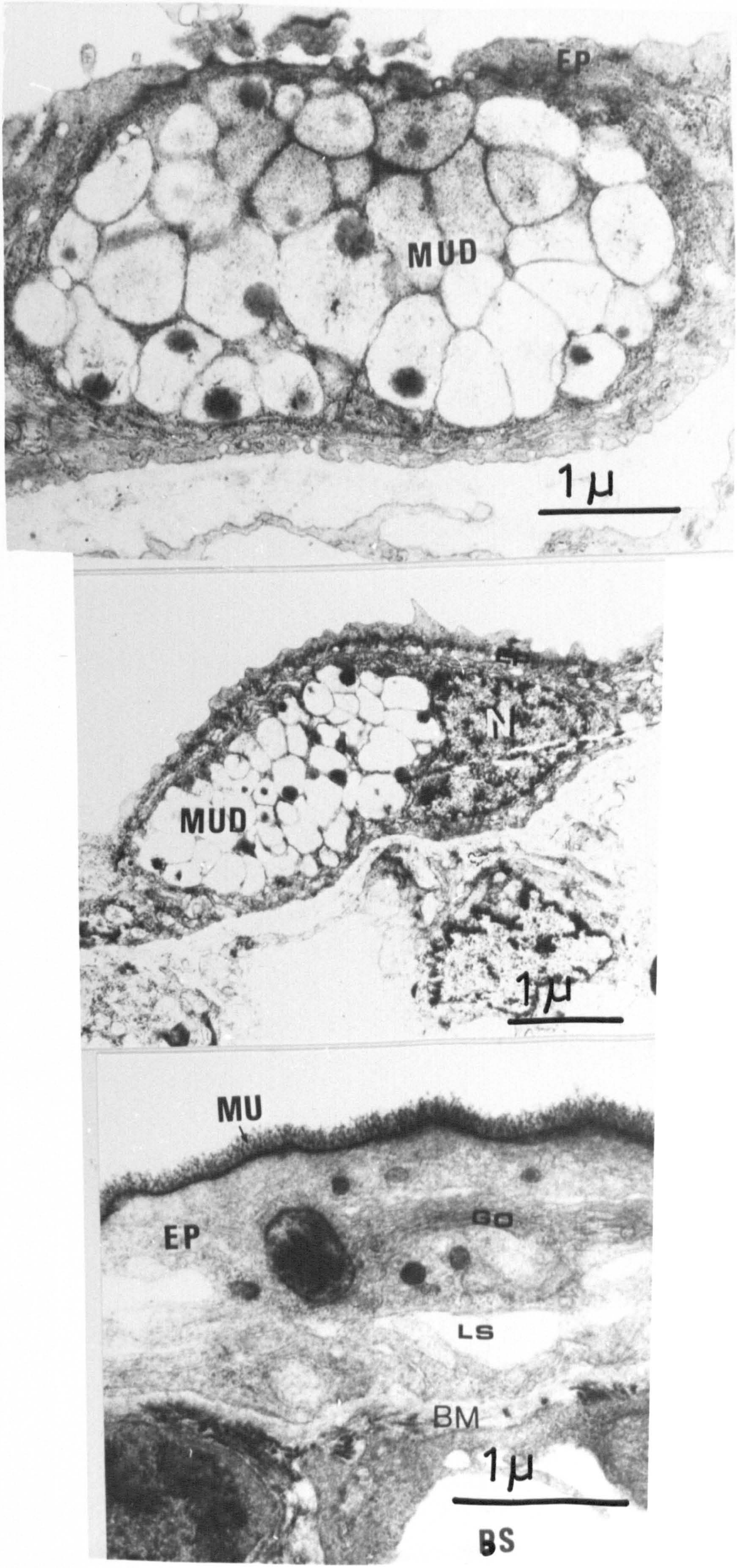
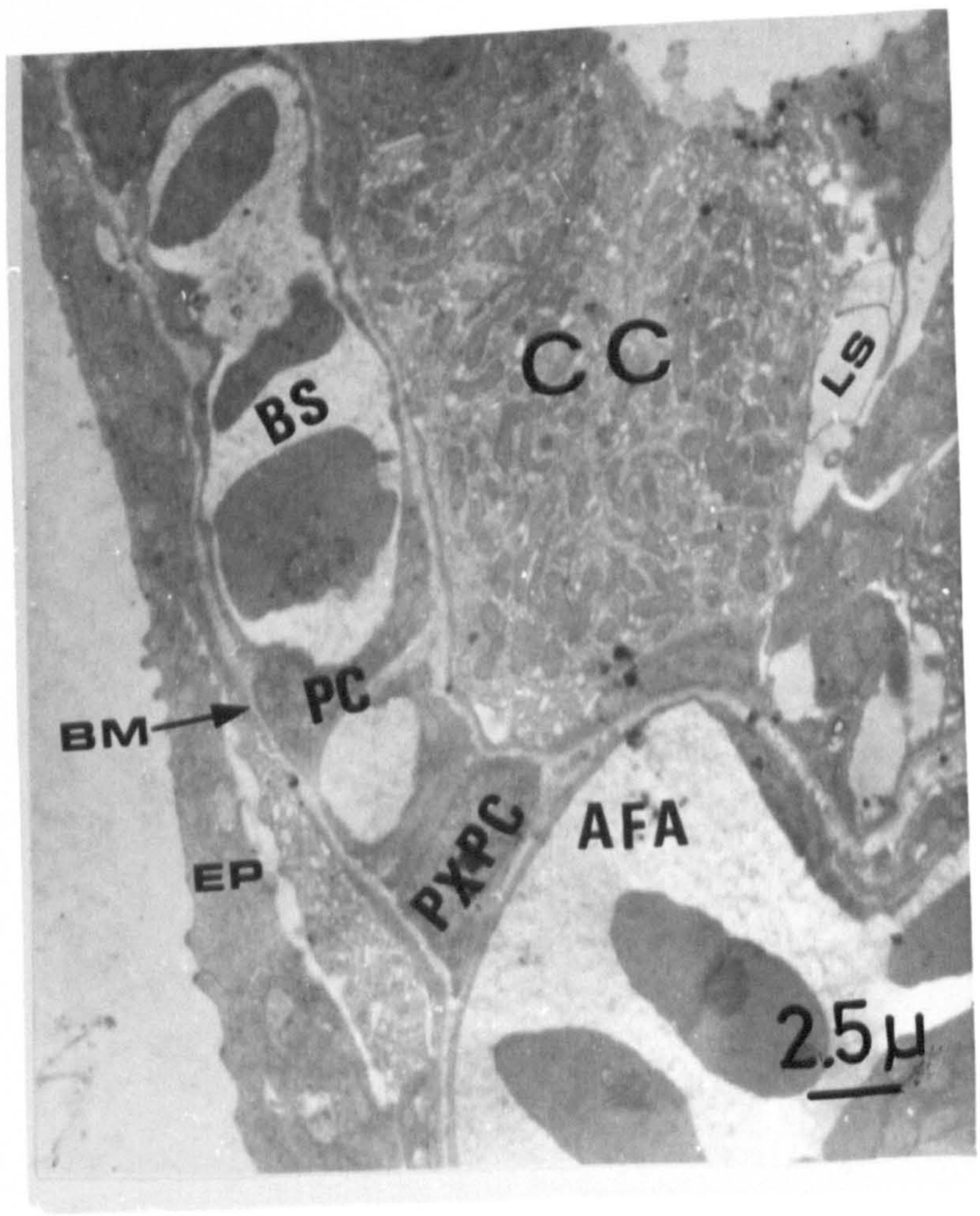

EP
MUD
1μ
N
MUD
1μ
MU
EP
GO
LS
BM
1μ
BS

PLATE 7

a. Electron micrograph of a section showing a part of central venous sinus of a gill filament (X 7,000)

b. Electron micrograph of a sagittal section through a gill filament and secondary lamella which are not isolated from the filament by the basal lamina of epithelial cells (EP), afferent filament artery (AFA), blood space (BS), Chloride cell (CC), Lymph space (LS) are also shown. (X 4,000)


2μ


BS
CC
LS
BM
PC
EP
PXPC
AFA
2.5μ

contact with mitochondria-rich cells (Pl. 7b). At the free edge of the chloride cell, the epithelium is discontinuous to form a pit (Pl. 8b). In the gill filament are present many phagocytic cells with deeply stained granules (Pl. 9a). In the primary epithelium are also present mitochondria-rich chloride cells and microridged epithelial cells. A central venous sinus in the core of the filament is also seen (Pl. 9a). Between the two blood channels there are found many collagen fibre bundles in the connective tissue layer of the gill filament (Plates 9a, 10).

4.3.4 MICROCIRCULATORY PATHWAY IN THE GILL FILAMENTS

Light microscopy of 1 μm ultratome sections reveals the following two vascular pathways in the gills:-

4.3.4.1 I. THE ARTERIO-ARTERIAL PATHWAY (RESPIRATORY)

The arterio-arterial pathway in the gills is mainly involved in conducting the venous blood to the secondary lamellae for gaseous exchange. It includes the afferent branchial artery, afferent filament artery, blood channels in the secondary lamellae, efferent filament artery and the efferent branchial artery.

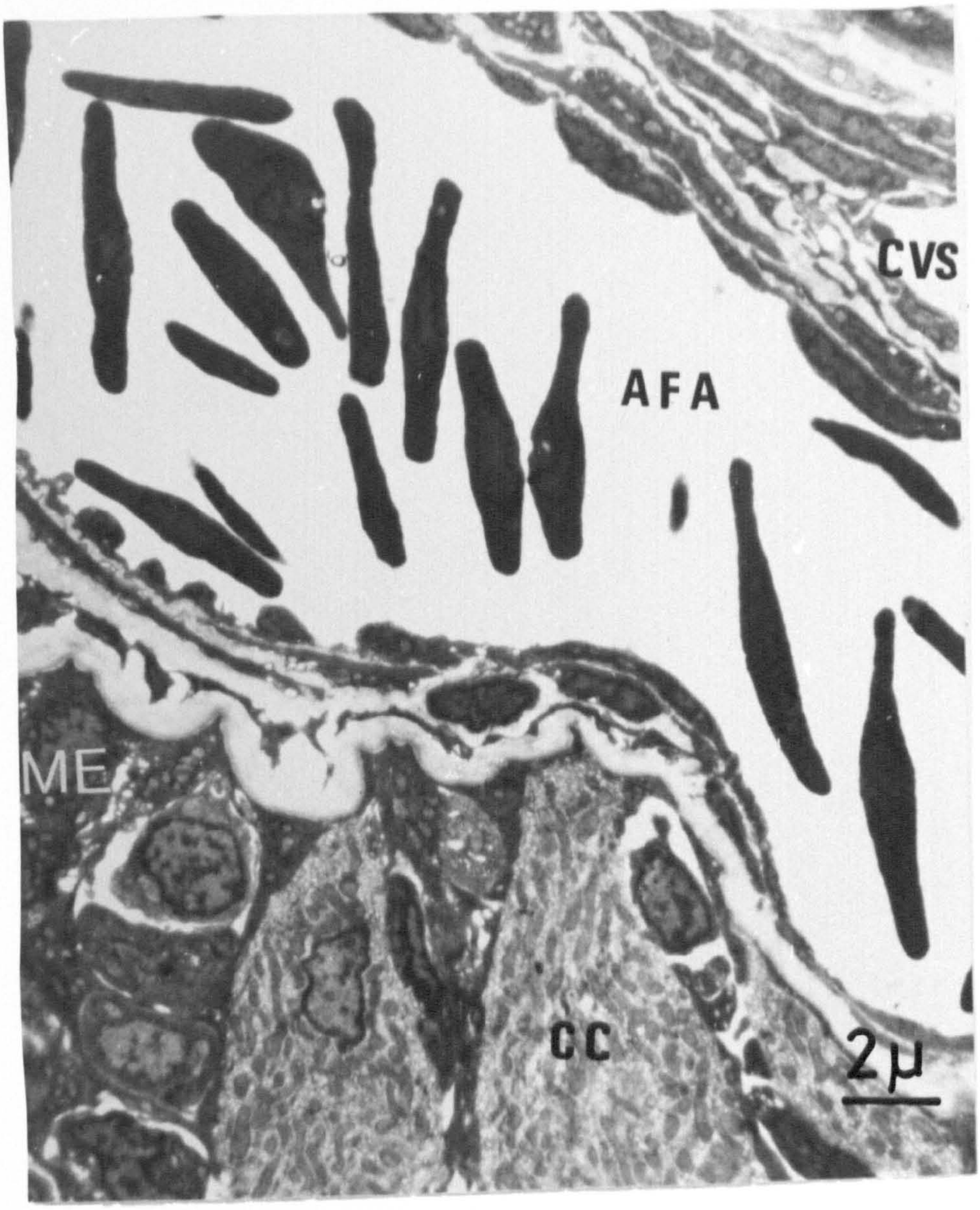
PLATE 8

a. Electron micrograph through the afferent filament artery (AFA). Chloride cell (CC); central venous sinus (CVS); mesenchyme cells (ME) are also shown.

(X 5,250)

b. Electron micrograph of a chloride cell showing apical aperture (arrow) surrounded by overlying extension of epithelial cells (EP). Note the large nucleus (N).

(X 10,600)


CVS
AFA
ME
CC
2μ

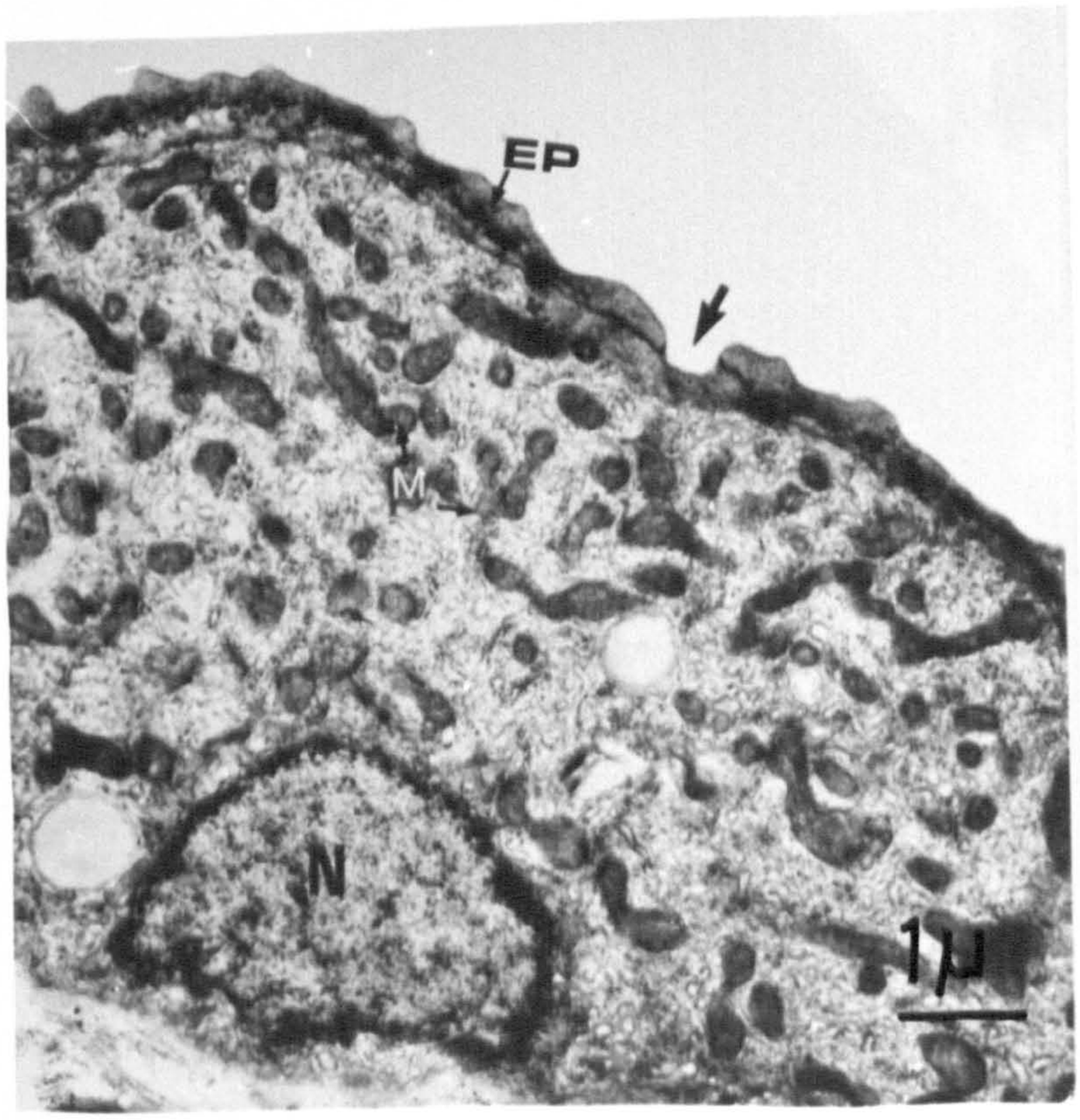

EP
M
N
1μ

PLATE 9

a. Electron micrograph of section through central venous sinus and secondary lamella, showing the continuous connection between the basal lamina of secondary lamella and central venous sinus of the gill filament. Basement membrane (BM); collagen (COL); granulocyte (G); pillar cell (PC), are also shown

(X 5,700)

b. Electron micrograph of section through the epithelium of the gill filament showing the chloride cell (CC) surrounded by two dark cells (DC) and mesenchyme cells (ME); and granulocyte cells (G). Central venous sinus (CVS); nucleus (N), are also shown.

(X 5,500)

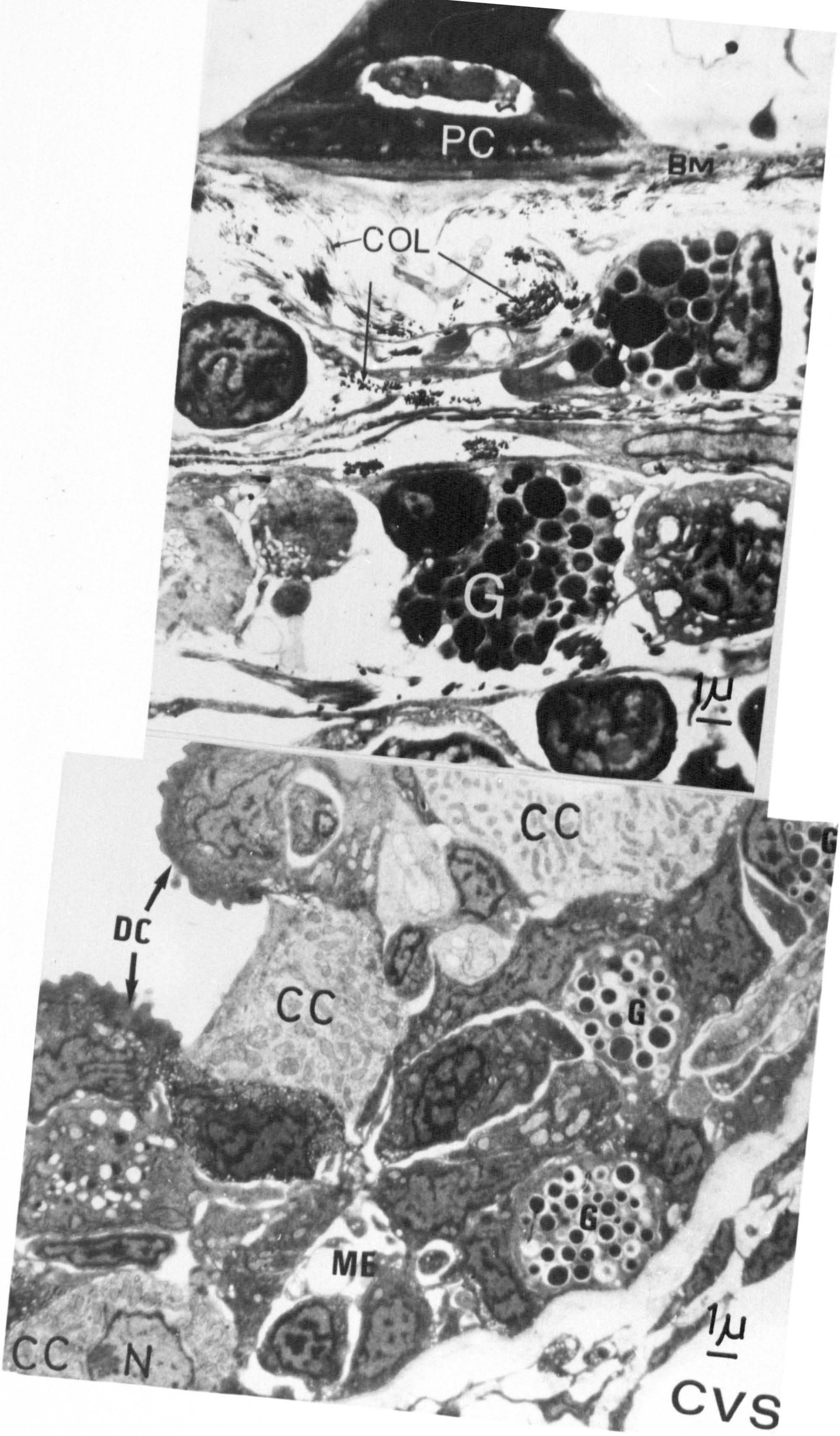
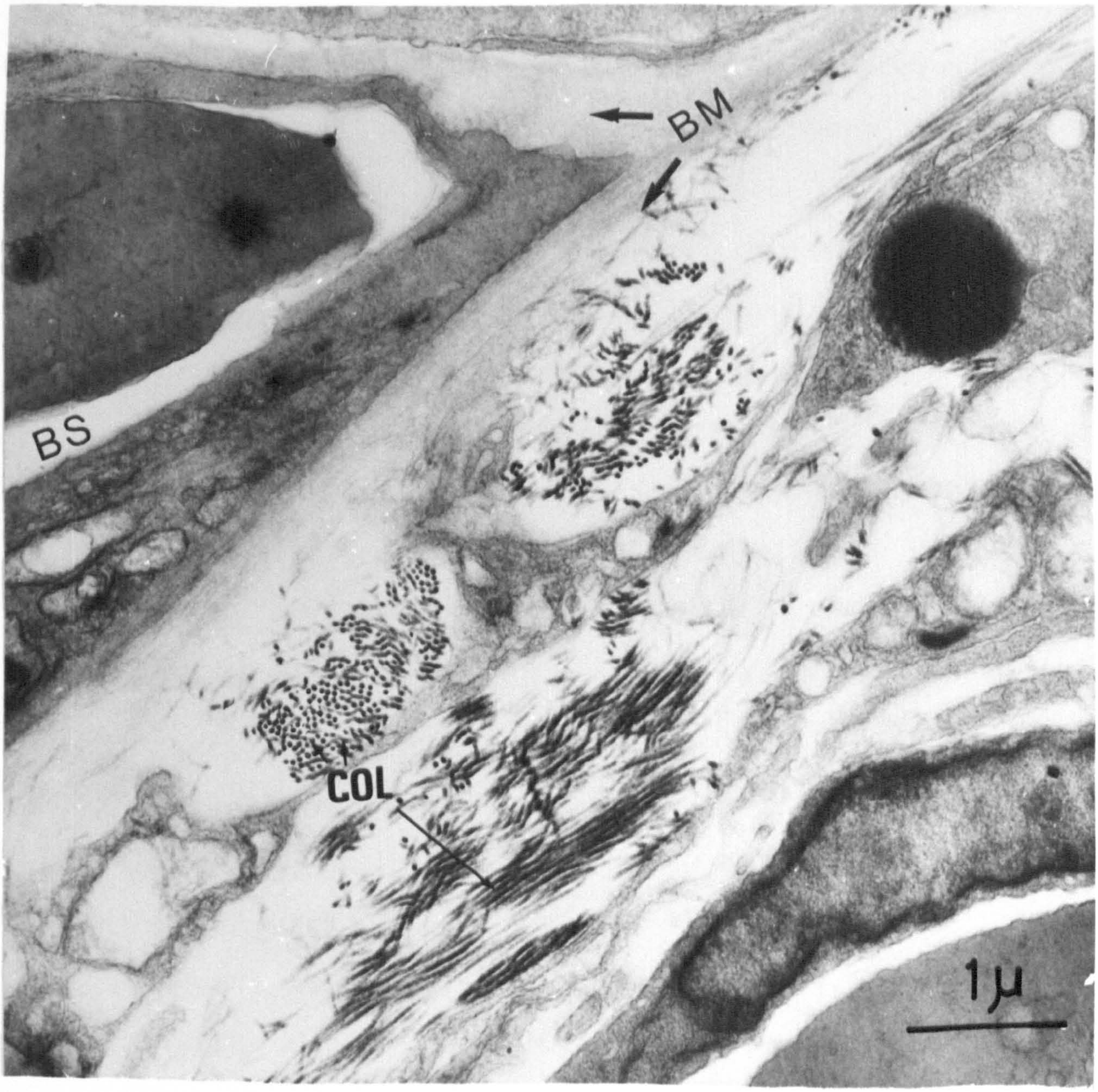

PC
BM
COL
G
1μ
D
CC
DC
CC
G
G
ME
CC
N
1μ
CVS

PLATE 10

Electron micrograph of transverse section through a gill filament and base of a secondary lamella which is not isolated from the filament by the basal lamina of epithelial layer (arrows). Collagen (COL); basement membrane (BM); blood space (BS) are also shown.

(X 23,250)


BM
BS
COL
1μ

Each of the gill arches contain afferent and efferent branchial arteries (Pl. 18b). The afferent branchial artery gives off an afferent filament artery to each gill filament of the two hemibranchs. Correspondingly the efferent branchial artery receives efferent filament arteries from the gill filaments. A schematic representation of the arterio-arterial pathway is shown in text-fig. 1. In each of the gill filaments, the afferent filament artery lies close to the cartilaginous gill ray, which is found on the afferent side of each filament. The efferent artery is located at a distance from this supporting structure. However, the distance between the gill ray and the efferent filament artery varies at different levels of each filament (Plates 11, 12) The wall of the afferent filament artery is formed of tunica intima, tunica media and tunica adventitia (Pl. 13a). The afferent filament artery gives off branches to each of the secondary lamellae. No valve which might serve to regulate blood flow in the lamellae has been observed at the origins of these two vessels (Pl. 13b).

The outer surface of the efferent filament artery is not smooth but also formed of the three usual layers (Pl. 13c). It receives oxygenated blood from the

TEXT FIG. 1

I. <u>THE ARTERIO-ARTERIAL PATHWAY (RESPIRATORY)</u>

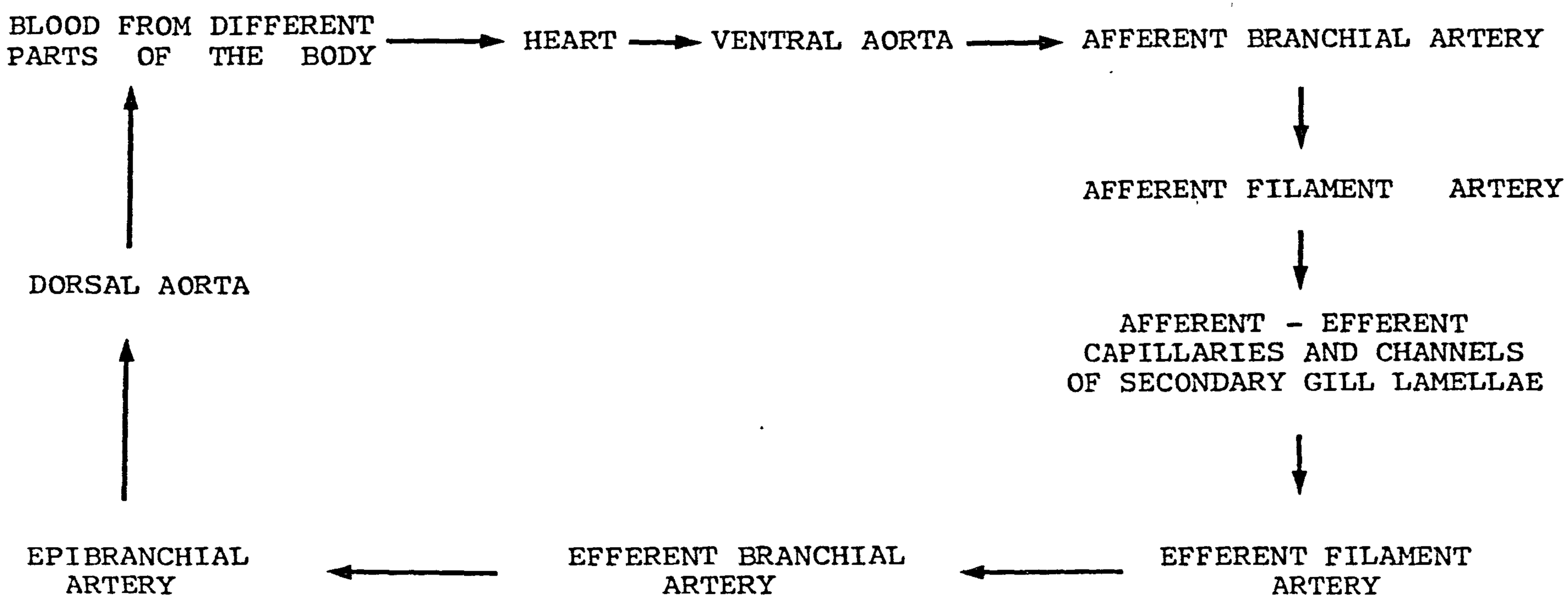
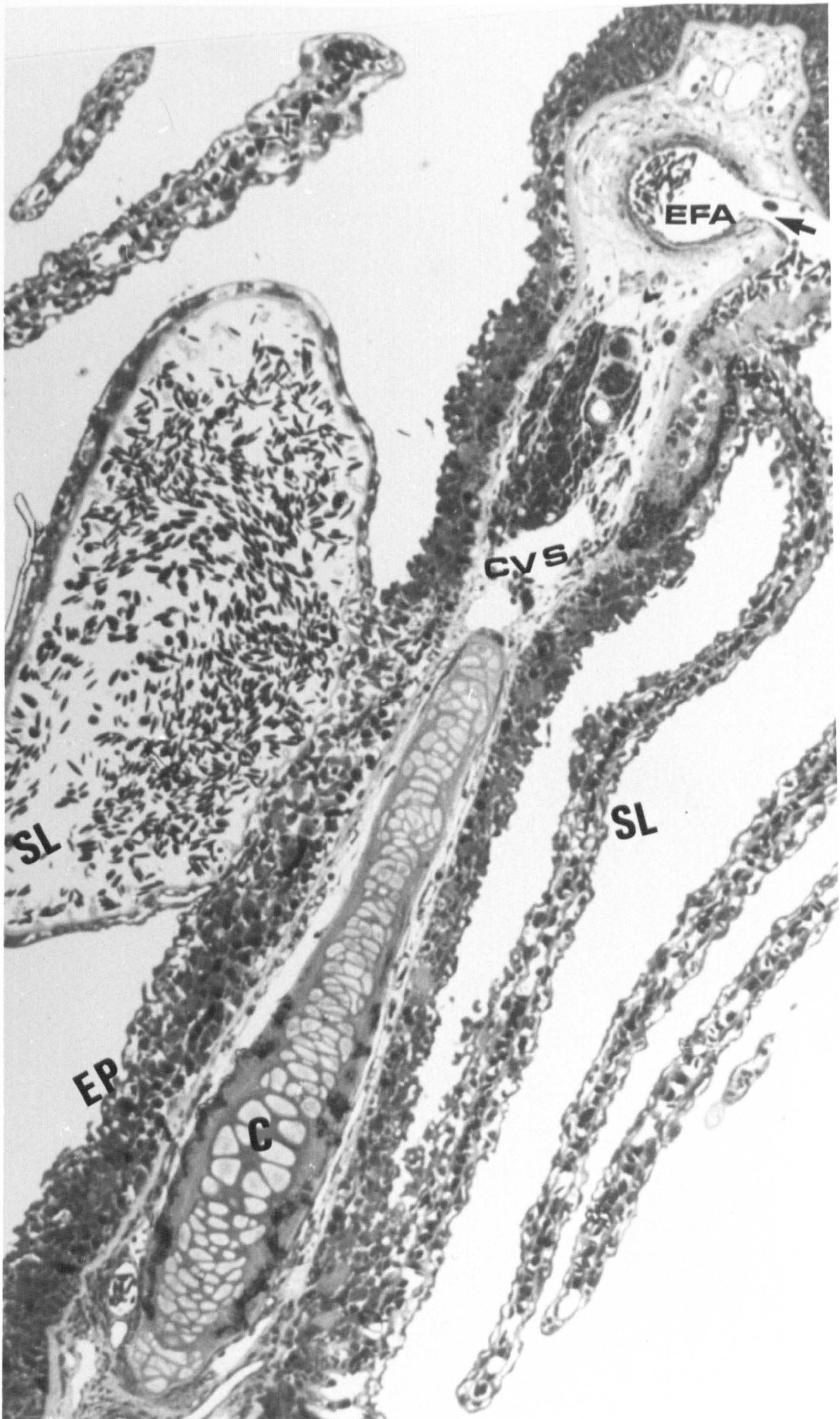


PLATE 11

Transverse section of a gill filament showing the afferent filament artery (AFA), efferent filament artery (EFA), cartilaginous gill ray (C) and the central venous sinus (CVS). Arrows indicate the direction of the blood flow to and from the secondary lamellae (SL). Epithelial layer (EP).



0.067mm

PLATE 12

Transverse sections of a gill filament from (a) tip; (b) middle and (c) base. Afferent filament artery (AFA); efferent filament artery (EFA); cartilaginous gill ray (C); secondary lamellae (SL); central venous sinus (CVS).

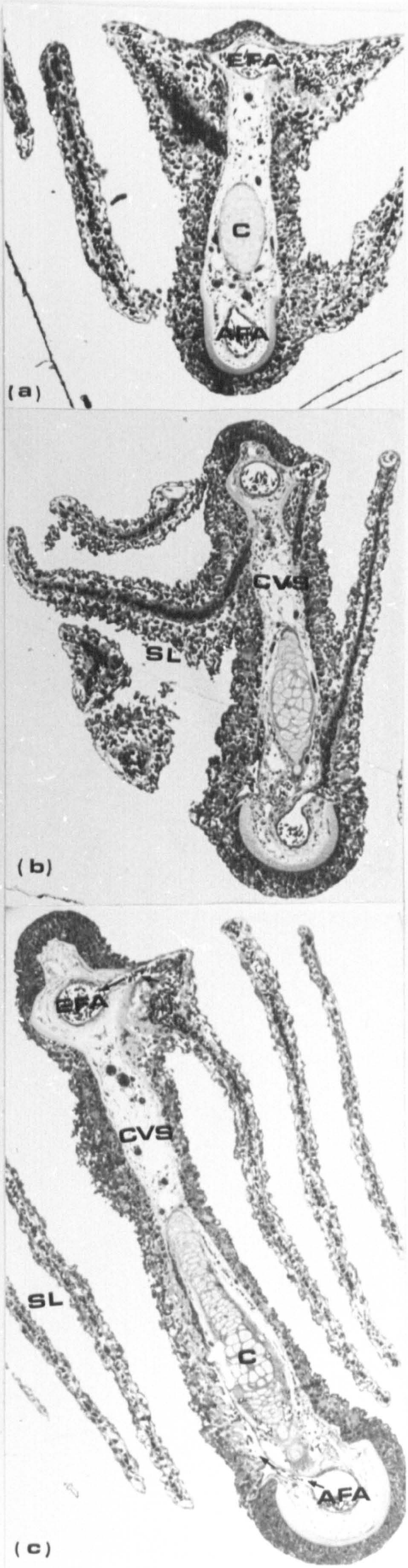

EFA
C
AFA
(a)
CVS
SL
(b)
EFA
CVS
SL
C
AFA
(c)
0.163mm.

PLATE 13

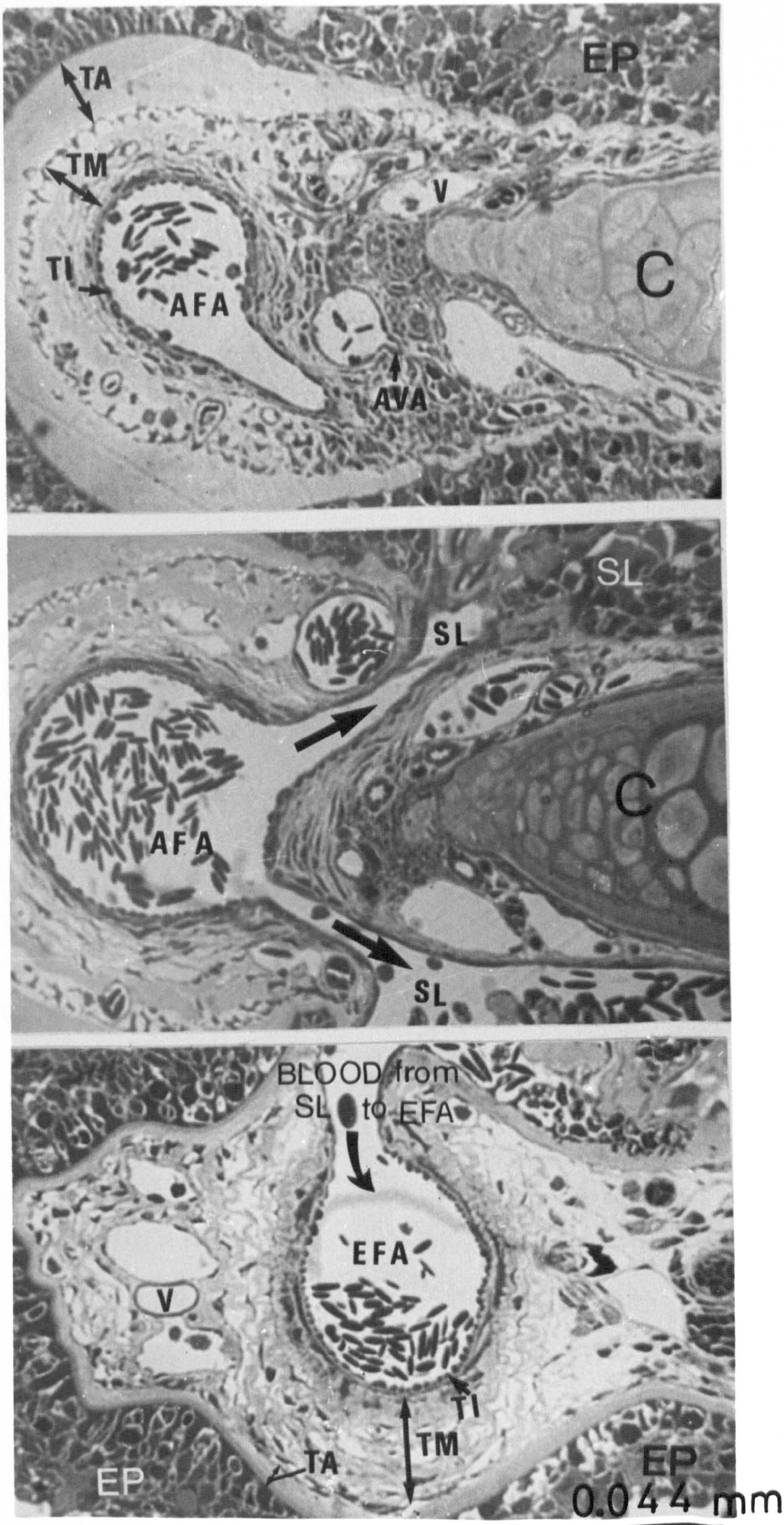
a. Part of a transverse section of a gill filament showing wall of the afferent filament artery (AFA). The arrows show layers of AFA's wall and thick arrow indicates the arterio-venous anastomoses (AVA). Tunica intima (TI); Tunica media (TM); Tunica adventitia (TA); Cartilaginous gill ray (C); epithelial layer (EP);

vein (V).

b. Transverse section of a gill filament showing the afferent filament artery (AFA) giving off two afferent secondary lamellar arteries as indicated by the arrows. Cartilaginous gill ray (C); Secondary lamellae (SL);

c. Part of a transverse section through a gill filament showing the nature of the wall of efferent filament artery (EFA). Epithelial layer (EP); vein (V); Tunica initima (TI); Tunica media (TM); Tunica adventitia (TA);

vein (V).


TA
TM
TI
AFA
AVA
V
EP
C
SL
SL
AFA
C
SL
BLOOD from
SL to EFA
EFA
V
TI
TM
TA
EP
EP
0.044 mm

secondary lamellae. As on the afferent side, no valve was observed at the places where the blood channels from the secondary lamellae open into the efferent filament artery (Pl. 13c).

4.3.4.2 II. THE ARTERIO-VENOUS PATHWAY (NUTRITIVE)

The arterio-venous pathway provides nutrition and oxygen to the gill filaments. It consists of the efferent filament artery, nutritive blood channels, central venous sinus, venules and the branchial veins.

The most important part of the arterio-venous system is the central venous sinus. In P. flesus the central venous sinus is an extensive network of blood spaces lying between the cartilaginous gill ray and the efferent filament artery (Pls. 13b, 14). This vascular network is more extensive at the base than in the middle and the tip regions of the gill filaments (Pls. 11, 12, 13b). The arterio-venous pathway is diagrammatically presented in text fig. 2.

The efferent filament arteries communicate with the central venous sinus through sphincter-like structures (Pls. 14, 15a). At certain places from each efferent filament artery, there arise many nutritive blood channels which traverse the inner core of the gill

TEXT FIG. 2

II. THE ARTERIO-VENOUS PATHWAY (NUTRITIVE)

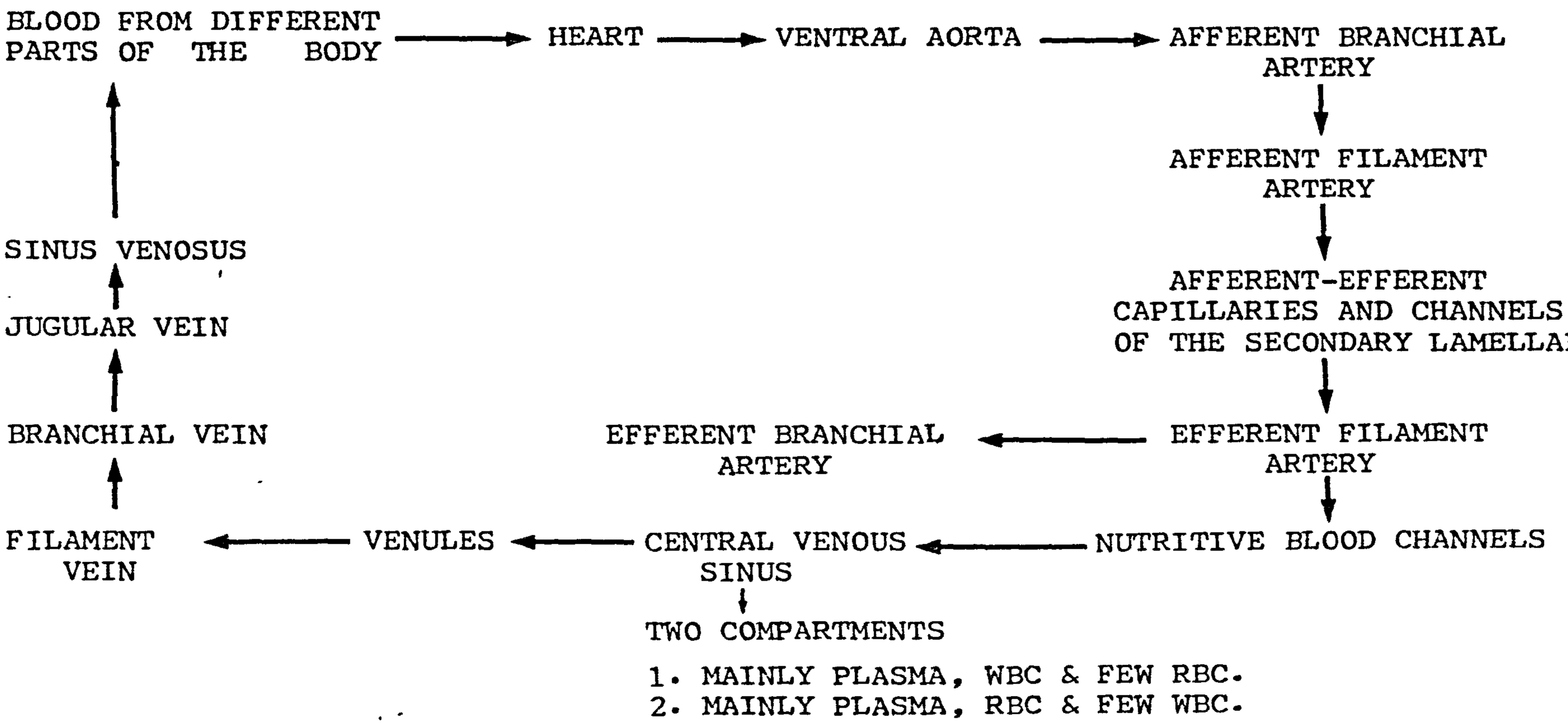


PLATE 14

Part of sagittal sections of a gill filament (a,b and c) showing the central venous sinus (CVS) between the cartilaginous gill ray (C) and the efferent filament artery (EFA). Note the origin of the nutritive blood vessels as indicated by the open arrows. Afferent filament artery (AFA); secondary lamellae (SL); epithelial layer (EP).

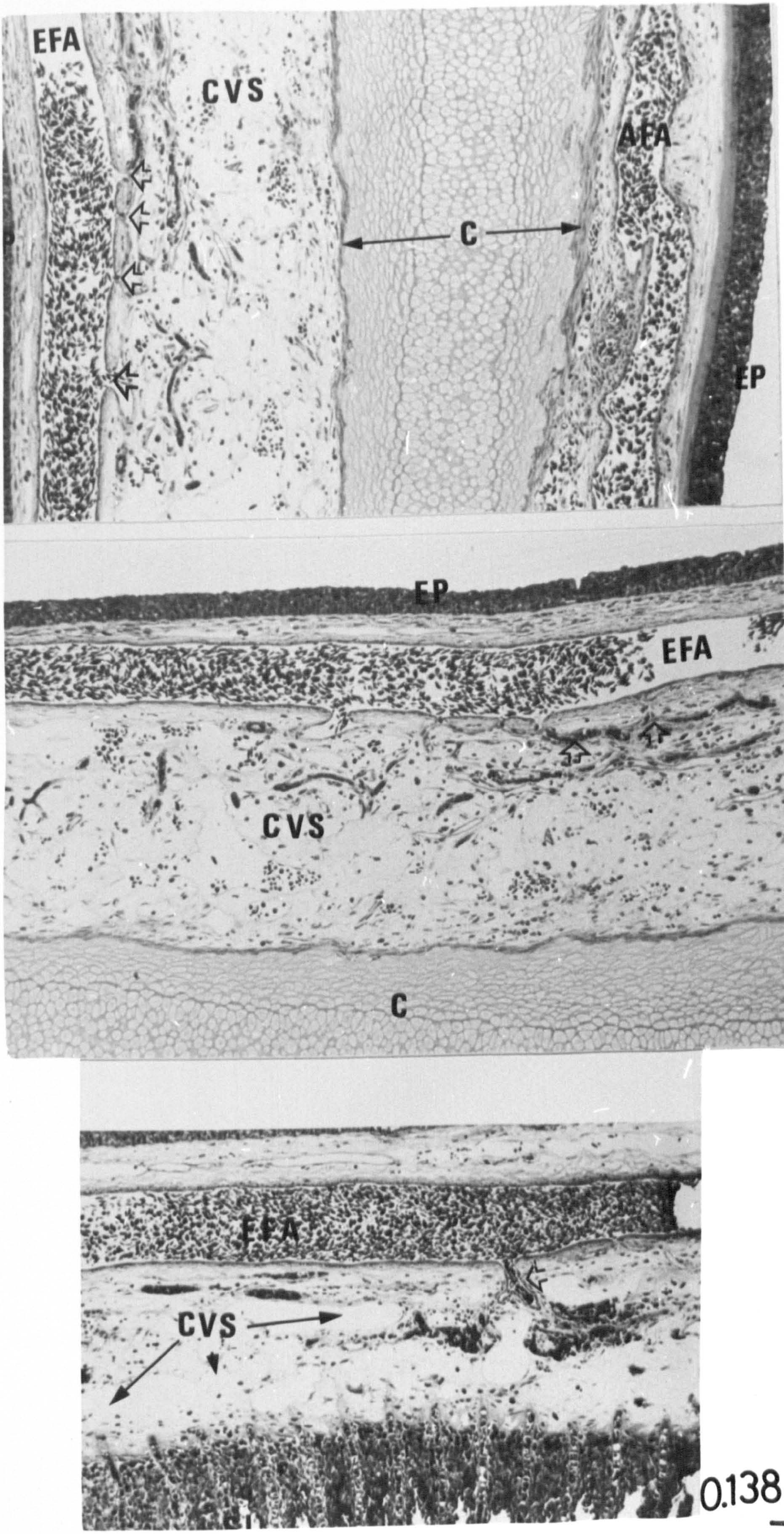
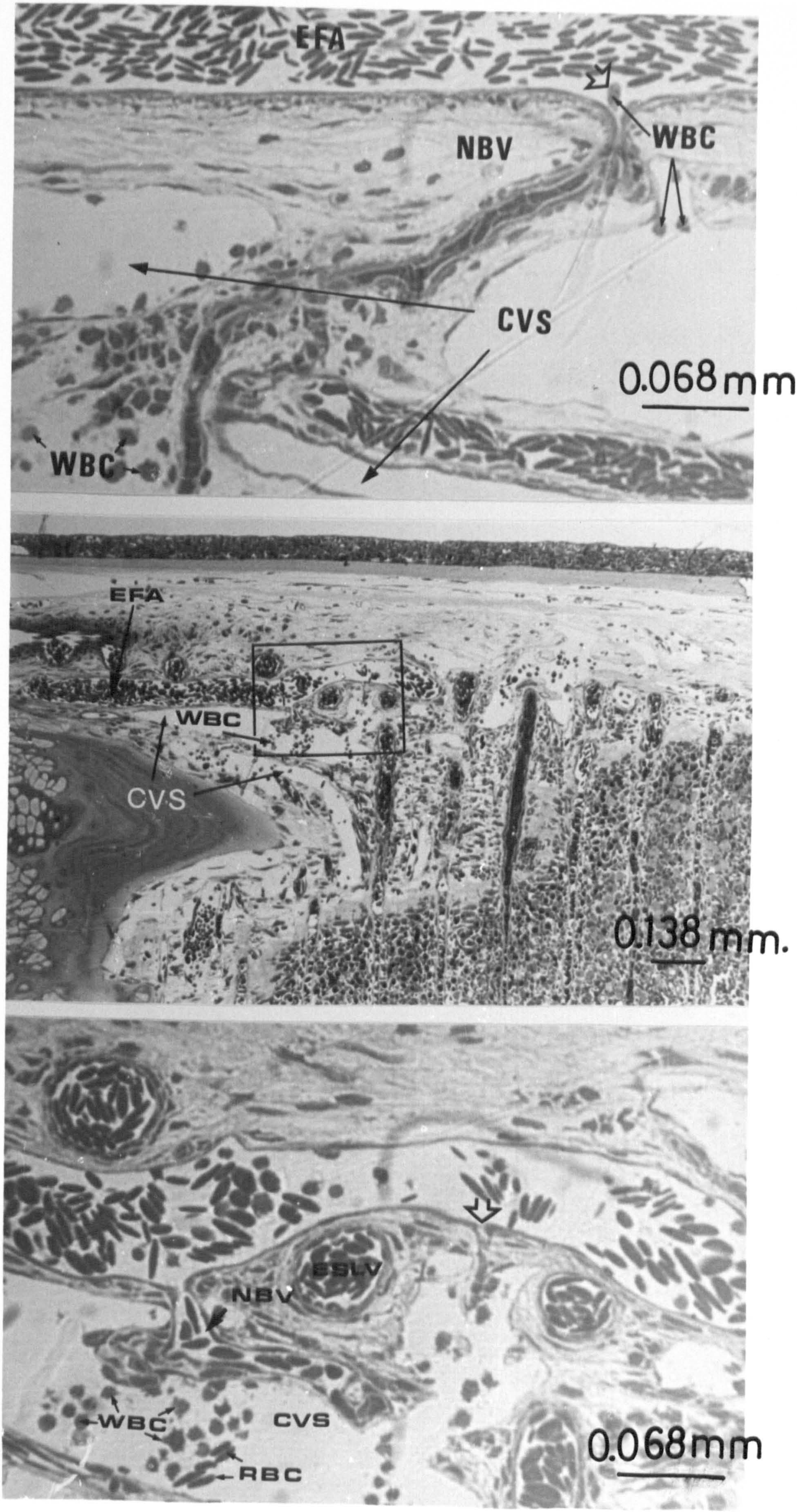

EFA
CVS
C
AEA
EP
EP
EFA
CVS
C
EFA
CVS
0.138 mm

PLATE 15

a. Part of a sagittal section of a gill filament. The open arrow indicates a sphincter-like opening of the efferent filament artery (EFA) into the central venous sinus (CVS) through the nutritive blood vessels (NBV). White blood cells (WBC) are also shown.

b. Part of a sagittal section of a gill filament showing the difference in number of white blood cells (WBC) and red blood cells (RBC) in the central venous sinus (CVS) and efferent filament artery (EFA).

c. Enlargement of area limited by black rectangle in Pl. 15b . The open arrow indicates a small opening which mainly allows WBC but few RBC to pass through into CVS. Also shown are nutritive blood vessels (NBV) and efferent secondary lamella vessels (ESLV).


EFA
NBV
WBC
CVS
0.068mm
WBC
EFA
WBC
CVS
0.138 mm.
ESLV
NBV
WBC
CVS
RBC
0.068mm

filaments. These fine blood channels are designated as nutritive blood channels. The central venous sinus in the gill filament of P. flesus is divided into two functional compartments. One compartment is mainly filled with plasma, RBC and few WBC and the other compartment is dominated by WBC and few RBC (Pl. 15a,c).

At certain places, the central venous sinus and veins do not contain any blood corpuscles. Arterio-venous anastomoses have also been observed towards the afferent side of the filament with a secondary artery which contains RBC's but appears to be separate from the AFA. However, a direct connection between afferent filament artery and this vessel has not been observed.

4.4 DISCUSSION

Fish gills present an interesting example of an organ system suited for respiratory, haemodynamic and osmo-regulatory functions. The respiratory capability of the secondary lamellae is directly proportional to their area and inversely proportional to the water-blood diffusion distances. As in other teleosts, the blood channels in the secondary lamellae of Platichthys flesus are formed by the flanges of adjacent pillar cells, which overlap each other to form a complete channel. The diameter of the marginal channel is larger than other blood channels of the secondary lamellae. This is an advantage for the fish as it is the marginal channels which are in contact with the ventilatory water that probably contains the most oxygen and therefore a large volume of blood becomes well-oxygenated before it reaches the efferent filament artery. The marginal channels are only partly lined by pillar cell flanges. The presence of osmiophilic bodies in the outer lining of these channels indicates its endothelial nature. This finding corroborates the previous reports of Newstead (1967), Hughes and Wright (1970) and Hughes (1977). Due to the resistance of the blood channels the blood flow velocity falls during its passage across each secondary lamella. Lower velocity of blood in the marginal channel gives sufficient time for the oxygen saturation of blood. Furthermore,

higher density of erythrocytes in the marginal channels enhances the oxygen carrying capacity of blood.

Series of pillar cells between the blood channels provide strength and prevent blood channels from collapsing. The basement membranes and columns with collagen fibres provide further support for the blood channels. The presence of mitochondria in the pillar cells indicates their greater metabolic activities. The contractile nature of the pillar cells was indicated by the presence of contractile materials (Bettex-Galland and Hughes, 1973). The energy required for the contraction of the pillar cells is perhaps provided by the oxidative phosphorylation of the fuels present in the pillar cells.

The diffusion distance in the secondary lamellae of P. flesus is small(3.42 μm) and is in the range of the values reported for purely aquatic teleostean fish. However, this value is lower than values obtained for air-breathing fish (Table 9).

The mucous glands present on the epithelia of filaments and secondary lamellae of P. flesus secrete mucus. Higher mucus secretion by the gills of flounders is an adaptation towards the sandy and muddy waters of sea and the estuaries.

Table 9. Summary of table showing the values on water-blood diffusion distance in the secondary lamellae of different fish species

FISH SPECIES	WATER-BLOOD BARRIER THICKNESS		REFERENCES
	Range µm	Mean µm	
1. WATER-BREATHING			
i. Elasmobranchs			
Scyliorhinus canicula (Smooth dogfish)	5.09-17.95	11.27	Hughes & Wright, 1970
Scyliorhinus stellaris (Spotted dogfish)	4.09-11.80	9.62	"
Squalus acanthius (Spiny dogfish)	3.42-29.18	10.14	"
Galeus vulgaris (Spiny dogfish)	2.27-23.01	9.87	"
Raia motagni (Spotted ray)	1.075-14.0	4.85	"
Raia clavata (Thornback ray)	2.875-10.05	5.99	"
ii. Teleosts			
Pleuronectes platessa (Plaice)	0.88-16.61	3.85	"
Solea solea (Sole)	1.35-3.360	2.80	"
Solea variegata (Sole)	1.97-21.12	5.55	"
Limanda limanda (Dab)	0.73-8.000	2.53	"
Microstomus kitt (Lemon sole)	0.61-43.45	3.23	"
Trachurus trachurus (Horse mackerel)	0.25-3.130	2.221	Hughes, 1970a
Scomber scomber (Mackerel)	0.60-3.625	1.215	"
Katsuwonus pelamis (Skipjack tuna)	0.24-1.906	0.598	"
Euthynnus affinis (Little tunny)	0.313-1.063	0.596	"
Thunnus albacares (Yellowfin tuna)	0.166-1.125	0.533	"
Onchorhynchus (Kitsuch Coho Salmon)	0.64-3.500	.....	"
Salmo gairdneri (Rainbow trout)	3.32-9.60	6.370	"
Porophrys vetulus (English sole	1.90-3.20	.....	Newstead, 1967
Carassius carassius (Crucian carp)	0.50-0.60	.....	Schulz, 1960
Tinca tinca (Tench)	1.60-3.50	2.473	Hughes, 1972
Barbus stigma (Punti)	0.80-3.20	.....	Munshi & Singh, 1968
Catla catla (Katla)	0.80-3.20	.....	"
Mystus cavasius (Cavasi tengra)	1.20-3.20	2.150	Singh, 1979
Mystus vittatus (Tengra)	0.90-2.00	1.380	"
Botia lohachata	.....	1.712	Sharma, 1980
Cirrhina mrigala (Mrigal)	.....	1.750	Roy, 1984
Rhinomugil corsula (Corsula)	.....	3.630	Mishra, 1984
Sicamugil cascasia	.....	2.57	Mishra, 1984
2. AIR-BREATHING			
Anabas testudineus (Climbing perch) (narrow trunked variety)	2.07-4.08	.....	Dube & Munshi, 1974
Anabas testudineus (Climbing perch) (Broad trunked variety)	15.00-20.00	.....	"
Macrognathus aculeatum (Mud eel)	0.50-3.00	1.54	Ojha, 1974
Clarias batrachus (Magur)	6.96-9.62	7.67	Singh, 1977
Channa gachua (Garai)	.....	2.40	Dandotia, 1978
Boleophthalmus boddaerti (Estuarine goby)	.....	1.43	Biswas, 1981
Platichthys flesus (Flounder)	0.80-14.80	3.42	Present study

The mucus produced by the gills is helpful in removing the sediments from the respiratory epithelium.

The mitochondria-rich chloride cells are useful in maintaining ionic balance between the blood and circulating water. Flounders inhabit brackish water of estuaries and face the problem of osmo-regulation. The chloride cells of flounders have to expend a lot of energy to cope with the problem created by the ionic imbalance between the body fluid and the ambient water. This assumption is supported by the higher concentration of mitochondria in the chloride cells. The presence of chloride cells in the primary epithelium close to the blood channels of the secondary lamellae embedded in the filament is interesting. From such observations, it may be suggested that blood passing through the secondary lamellae performs two functions. The blood channels of the secondary lamellae embedded in the primary epithelium of the filament plays a dominant role in ionic exchange with the help of chloride cells and the blood channels present in the free part of the secondary lamellae function in gaseous exchange.

The existence of two independent vascular pathways in fish gills has often been a matter of controversy since their description by Muller (1839) and Reiss (1881). The present findings support the existence

of two independent vascular pathways in the gills of Platichthys flesus. The arterio-arterial pathway (respiratory) is of a typical teleostean type (Hughes and Grimstone, 1965; Vogel, Vogel and Schlote, 1974; Laurent and Dunel, 1976). Steen and Kruysse (1964) described the shunting of blood from the afferent to the efferent filament artery through the central venous sinus. However, the presence of such a direct shunt has been doubted on both morphological and haemodynamic grounds (Hughes, 1972, 1979). The present study confirms the absence of such a direct pathway in P. flesus but evidence has been obtained for the passage of blood from the efferent filament arteries to the central venous sinus through very small nutritive blood vessels. The origins of these vessels from the efferent filament arteries are guarded by sphincter-like structures which may help in the regulation of blood flow to the central venous sinus.

The view that in fish gills there exist separate vascular pathways through which few erythrocytes circulate has been suggested on a number of occasions. These include the intraepithelial "lymphoid" spaces (Hughes and Wright, 1970; Hughes, 1980a) and an independent lymphatic system connected to the central venous sinus (Richards and Fromm, 1969). Several morphometric studies have indicated that

the haematocrit value of different vascular pathways within the gills may also vary (Hughes, 1979, 1980b). Scanning electron micrographs (Vogel, Vogel and Schlote, 1974; Vogel, Vogel and Pfautsch, 1976) have shown that arterio-venous anastomoses between filament arteries (mainly efferent) and the central venous sinus are guarded by endothelial cells with finger-like projections into the vessel lumen and it has been suggested that such structures would restrict the flow of red cells into the CVS (Hughes, 1980b). Furthermore, plasma might pass directly through the endothelium as has been suggested for other parts of the fish circulatory system (Soivio and Hughes, 1978). The existence of an independent vascular system largely devoid of red blood cells may, therefore, be a common feature of fish gills. In the flounder, the presence of such a compartment filled with white blood cells may be a special adaptation related to their habitat in the mud of estuaries and thus enable them to combat bacterial and fungal infections as has also been supposed for the phagocytic white cells in the lymphoid spaces of the secondary lamellae. The presence of such cells within the epithelium of the secondary lamella and gill filaments could form a defence mechanism similar to that provided by the alveolar macrophages in the mammalian lung (Hughes, 1980b).

The main supporting structure within the gill filaments of teleosts is provided by the gill ray which is situated towards the afferent filament artery. The efferent side has no such support and the presence of the central venous sinus filled with plasma between the cartilaginous ray and the efferent filament artery may act as a hydroskeleton to provide additional support for this part of the filament.

It has also become clear from both physiological and morphological studies that the condition of the vascular circulation in fish gills is very sensitive to environmental conditions. Consequently, the precise conditions under which fixation of gill material is carried out can affect the appearance of the vascular pathways. Although the present description is based upon several specimens which were fixed in similar conditions, the possibility that the nature of the pathways observed could change cannot be ruled out on the basis of the present study.

CHAPTER 5

MORPHOMETRICS OF THE GILLS OF PREMETAMORPHIC AND ADULT STAGES OF FLOUNDER, PLATICHTHYS FLESUS (L.)

Chapter 5 : MORPHOMETRICS OF THE GILLS OF PREMETAMORPHIC AND ADULT STAGES OF PLATICHTHYS FLESUS (L)

5.1 INTRODUCTION

Except for some air-breathing species, fish are primarily water-breathers using gills to extract sufficient oxygen from water to supply their aerobic metabolic requirements. The functioning of fish gills in oxygen uptake depends on their effective surface area and thickness of the water/blood diffusion barrier. In recent years, attempts have been made to measure quantitatively these dimensions of the respiratory organs of fish. Earlier measurements of gill surfaces have been relatively few in number and general in treatment. Pütter (1909) presented his data on gill measurements for four specimens of Scorpaena. However, the study of Price (1931) on the small-mouthed black bass, Micropterus dolomieu seems to be the first detailed study on the gill surface of a single species throughout the life cycle and to correlate such measurements with body size. But the methodology used in this study introduced some inaccuracies because the profile and dimensions of individual secondary lamellae were not determined as a triangular approximation was used in calculating the area of single secondary lamellae. Subsequently, many studies were made (e.g. Gray, 1954; Byczkowska-Smyk, 1957, 1958, 1959a, b, 1961, 1962) to

evaluate the gill dimensions in various fish species until Hughes (1966) put forward a new method for the determination of gill area in fish. He considered almost all the parameters which contribute to the total gill area and presented data on gill measurements for fourteen species of British fish and an Antarctic haemoglobinless icefish, Chaenocephalus. The data on total gill area measurements were obtained following the equation:

Total gill area = (2L/d) bl where L = total filament length, 1/d = frequency of secondary lamellae per mm of one side of filament, and bl = average bilateral surface area of a secondary lamella

According to Hughes (1966), more active fish have larger gill areas and better gaseous exchange capabilities in comparison with those of more sluggish ones. This is due to greater filament length and a larger number of secondary lamellae. Hughes (1966) pointed out that the resistance to flow relative to area is less in sluggish fish due to more widely spaced secondary lamellae. Hughes, Dube and Munshi (1973) and Hughes, Singh, Guha, Dube and Munshi (1974) established a quantitative relationship between body weight and various gill parameters for the two Indian air-breathing fish, Anabas testudineus and Heteropneustes

fossilis. They pointed out the impact of air-breathing on the various gill parameters of these fish. These measurements were based on the unweighted method. Muir and Hughes (1969) advocated a weighted method for the determination of gill dimensions in fish. With the help of this method, they estimated the total area of the secondary lamellae in the gills of skipjack tuna (Katsuwonus pelamis), yellowfish tuna (Thunnus albacares), and bluefin tuna (Thunnus thynnus). Quantitative relationships were obtained for body weight and various gill parameters of these species. For all three species, the slope of the regression line relating total gill area and body weight was found to be about 0.85, a value close to that (0.81) obtained for oxygen consumption and body weight for a large number of other teleost species. Since then, this method has been widely used for gill measurements (Hughes, 1970, 1972; Hughes and Gray, 1972). Subsequently, Hughes and Morgan (1973) revised the weighted method and presented a new one for the detailed measurement of gill dimensions. In the new method, they considered the dimensions of the gill filaments on both anterior and posterior hemibranchs of each of the four gill arches. They also pointed out the effect of different sampling methods on the gill dimension measurements and suggested using corresponding regions for sampling the frequency and average bilateral surface area of the secondary lamellae on each of the selected

filaments. Since then many gill measurements have been carried out on adult specimens of several species, e.g. the oceanic sunfish, Mola mola (Adeney and Hughes, 1977), the Pacific deep-sea fish, Synaphobranchus affinis, Serrivomer sector, Gonostoma elongatum, Bathythphlops marionae, Barbourisia rufa, Xyelacyba myersi (Hughes and Iwai, 1978), the coelacanth Latimeria chalumnae (Hughes, 1980), the air-breathing catfish, Clarias batrachus (Munshi, Ojha and Sinha, 1980) and the amphibious mudskipper, Boleophthalmus boddaerti (Niva, Ojha and Munshi, 1981) using the aforesaid method or with slight modifications. Furthermore, Hughes (1984) has discussed the practical problems involved in measuring gill areas of fish especially those which arise from different sampling methods and shrinkage following different treatments using a variety of fish species. However, little is known of the effect of growth on the gill dimensions of the larval and adult stages of flatfish (De Silva, 1974).

The present chapter of the thesis is an endeavour to elucidate the effect of growth on the various gill parameters of premetamorphic and adult stages of the flounder, Platichthys flesus. The data obtained for the gill measurements may also be helpful in evaluating the capability of this species to withstand the hypoxic water of the estuaries during low tide.

5.2 MATERIALS AND METHODS

5.2.1 ANIMALS

Platichthys flesus is a bottom dwelling flatfish belonging to the family Pleuronectidae of the order Heterostomata. This species is common in estuaries and harbours and often goes upriver to freshwater. It buries itself in the sand or mud with only the eyes and mouth above the surface. Although the flounder can live in freshwater and prefers dwelling in the brackish water of estuaries, it migrates to the sea to spawn.

5.2.2 EXPERIMENTAL FISH AND THEIR MAINTENANCE

Experimental fish used for gill dimension measurements were divided into two groups. The first group consisted of the larval stages with a body weight range of 0.001-0.05g and the second group included adults, the total weight range of the second sample being 0.05-200g. The larval stages were obtained by culturing the flounder in the laboratory using specimens reared in seawater at 12^{o} and $13^{o}C$ for morphometric measurements at the Marine Biological Association Laboratory, Plymouth. The adult fish supplied by MBA Laboratories were maintained in seawater at $12\text{-}13^{o}C$.

5.2.3 METHODS APPLIED FOR THE GILL DIMENSION MEASUREMENTS

Fresh body weights of pre-metamorphic and adult stages were determined with the help of an electronic balance and Mettler microbalance respectively. The fish were anaesthetised in MS222 (0.01g/L), the opercula removed and the fish were fixed in Bouin's fluid in seawater. After 24-48 hours of fixation, all the four gills of both sides were dissected out carefully, washed in 70% alcohol and measurements were made under a dissecting binocular microscope with an ocular micrometer fitted in one of its eye pieces. The methodology of Hughes and Morgan (1973) was followed with some modifications for the measurements of the following gill parameters in order to obtain total gill area.

5.2.3.1 Filament length

Total gill area is directly proportional to the total filament length and therefore it is one of the important parameters in gill dimension measurements. The method adopted here has already been described (Hughes and Iwai, 1978; Hughes, 1984). Each gill arch was divided into different sections. An average filament length of each section of both hemibranchs was determined separately by dividing the sum of the first and the last filament of each section by two. The total filament length for each of the sections was

determined by multiplying the average filament length and the number of filaments in that section. Filament lengths from different points of the two hemibranchs were measured in order to examine closely the gill architecture of P. flesus.

5.2.3.2 Secondary lamellar frequency

In adult fish, the number of secondary lamellae per millimeter on one side was determined for the base, middle and tip of the sampled filament. In pre-metamorphic stages, the frequency of secondary lamellae per millimetre was determined from thick sections obtained from sampled filaments. The same treatment was given to material from different developmental stages and hence any artefacts that may be introduced, such as shrinkage, are assumed to have been identical for all stages.

5.2.3.3 Average bilateral surface area

For average bilateral surface area, hand cut sections were obtained from the aforesaid samples used for the determination of secondary lamellar frequency. The profile of each sampled secondary lamella was traced on paper of uniform thickness and an average bilateral surface area calculated by weighing the pieces of tracings and comparing them with the weight of the paper of known surface area.

5.2.3.4 Total gill surface area of various sections

The total lamellar area of each of the sections was determined from the equation:

$$A = (L \times n \times bl)$$

where, A = total gill surface area of a sampled section, L = total filament length of that section, n = secondary lamellae frequency per unit length on both sides of the gill filament and bl = average unilateral surface area of the samples of secondary lamellae.

5.2.3.5 Weighted values for average bilateral surface area and frequency of secondary lamellae

The number of secondary lamellae of the sections was summed and divided by the total filament length to obtain a weighted value for secondary lamellae/mm. Similarly the summed total surface area of the sections divided by the total number of secondary lamellae gave a weighted value for average bilateral area.

5.2.3.6 Total gill area for the whole fish

The total gill area of each of the four gill arches was obtained separately by multiplying their respective total filament length by the product of a weighted average bilateral surface area and the weighted frequency of secondary lamellae/mm on both sides of

the filaments. The surface area for each of the four arches was summed and doubled to give the total gill surface area for the entire fish.

5.2.4 STATISTICAL COMPUTATION

The general equation:

$$Y = aw^b \text{ or } \text{Log } Y = \text{Log } a + b \text{ Log } W$$

was used for various allometric relationships, where Y is the parameter analysed; W is the wet weight; a and b are respectively the intercept and the slope of the regression line. The data obtained from the measurements were analysed by linear logarithmic transformations, using the least square regression method. Hewlett Packard 9810A computer was used for data computation.

5.3 RESULTS

The upper and lower opercular chambers of Platichthys flesus contain four pairs of gills. The upper chamber has a larger volume and contains better developed gills in comparison to those of the smaller lower chamber (Figs.26,27).Furthermore, there are differences in detail between corresponding regions of the upper and lower gill systems. Changes in various gill parameters with increase in body weight showed well defined trends. The data obtained for different weight groups of pre-metamorphic larval (0.001-0.05g) and adult (0.05-200g) fish are summarised in Tables (10, 11).

Regression analyses were carried out between body weight and various gill parameters and relationships based on the allometric equations are given separately for larval and adult flounders in Table 12. From these relationships, dimensions for standard 0.001, 0.01, 0.05 (larval), 1, 10 and 100g (adult) fish were calculated together with 95% confidence limits and standard deviations for the slope and intercept values (Sb, Sa) Tables 13, 14.

5.3.1 RELATIONSHIP BETWEEN BODY WEIGHT AND TOTAL FILAMENT LENGTH

The detailed measurements of filament length at

Table 12. Summary of table showing relationships between body weight and various gill parameters of the gills of Platichthys flesus L., before and after metamorphosis. Correlation coefficients are also given.

	BEFORE METAMORPHOSIS		AFTER METAMORPHOSIS	
Gill parameters	Equations	Correl. coeff.	Equations	Correl. coeff.
Total filament length (mm)	Log L = 3.5445 + 1.0985 logW $L = 3503.483W^{1.0985}$	0.9141	Log L= 2.6127 + 0.502 log W $L = 409.945\ W^{0.502}$	0.9841
Average number of sec. lamellae /mm	Log n = 1.5985-0.0522 log W $n = 39.6760\ W^{-0.0522}$	0.8767	Log n = 1.6611-0.0641 logW $n = 45.8212\ W^{-0.0641}$	0.9218
Bilateral area of an av . sec. lamella (mm^2)	Log bl= 0.8268+1.1832 log W $bl= 0.1490\ W^{1.1832}$	0.9242	Log bl= 1.4684+0.3859 log W $bl= 0.0340\ W^{0.3859}$	0.9734
Total gill area (mm^2)	Log A= 4.2764+2.2128 log W $A= 1.8896\ W^{2.2128}$	0.9705	Log A= 0.8425+0.8240 log W $A= 14.3700\ W^{0.824}$	0.9887
Total gill area (mm^2)/g body weight	Log G= 4.2869+1.2178 log W $G= 1.9361 \times 10^4\ W^{1.2178}$	0.9104	Log G= 2.7600-0.1550 log W $G= 575.4329\ W^{-0.1550}$	0.9220

Table 13. Summary of measurements showing the gill area and its component parameters for specimens of 0.001, 0.01 and 0.1g larval stages together with 95% confidence limitS computed by using logarithmic transformations . Standard deviation for the slope (Sb) and the intercept (Sa) of the regression lines are also given.

LARVAL STAGES (BEFORE METAMORPHOSIS)

Gill parameters	0.001g	95% conf. limit	0.01g	95%conf. limit	0.1g	95%conf. limit	Sb	Sa
Total filament length (mm)	1.7746	3.2135 0.9800	22.2619	28.7330 17.2482	279.2705	608.0915 128.2570	0.1303	1.9251
Average no. of secondary lamellae/mm	56.8746	58.8919 54.9265	50.4417	51.2029 49.6918	44.7364	46.8271 42.3790	0.00775	1.0392
Bilateral area of an av . sec. lamella (mm^2)	0.000042	0.000076 0.000023	0.000641	0.000828 0.000496	0.00977	0.021335 0.004477	0.1307	1.9293
Total gill area (mm^2)	0.0043	0.0085 0.0022	0.7091	0.9456 0.5317	115.7514	278.4751 48.1134	0.1470	2.0937
Total gill area(mm^2)/g body weight	4.3018	8.4416 2.1922	71.0243	93.8903 484.7141	1172.6368	2836.8829 484.7141	0.1479	2.1035

Table 14. Summary of measurements showing the gill area and its component parameters for specimens of 1, 10 and 100g adult stages together with 95% confidence limits computed by using logarithmic transformations. Standard deviation for the slope (Sb) and the intercept (Sa) of the regression lines are also given.

ADULT STAGES (AFTER METAMORPHOSIS)

Gill parameters	1g	95% conf. limit	10g	95% conf. limit	100g	95% conf. limit	Sb	Sa
Total filament length (mm)	409.9550	468.9570 358.3590	1302.7571	1478.0560 1148.2484	4140.0088	5086.1600 3369.8650	0.0242	1.0647
Average no. of secondary lamellae/mm	45.8212	47.6915 44.0282	39.5374	41.0505 38.0801	34.1153	36.2694 32.0891	0.0072	1.0188
Bilateral area of an av . sec. lamella (mm^2)	0.0340	0.0389 0.0297	0.0827	0.0939 0.0729	0.2011	0.2472 0.1636	0.0243	1.0649
Total gill area (mm^2)	638.9047	769.1171 530.7373	4259.9105	5070.1400 3579.1640	28403.0440	37726.9655 21383.4569	0.0338	1.2354
Total gill area (mm^2)/g body weight	575.4329	823.8749 401.9092	402.7282	564.0686 287.5399	281.8573	488.1736 162.7363	0.0646	1.18213

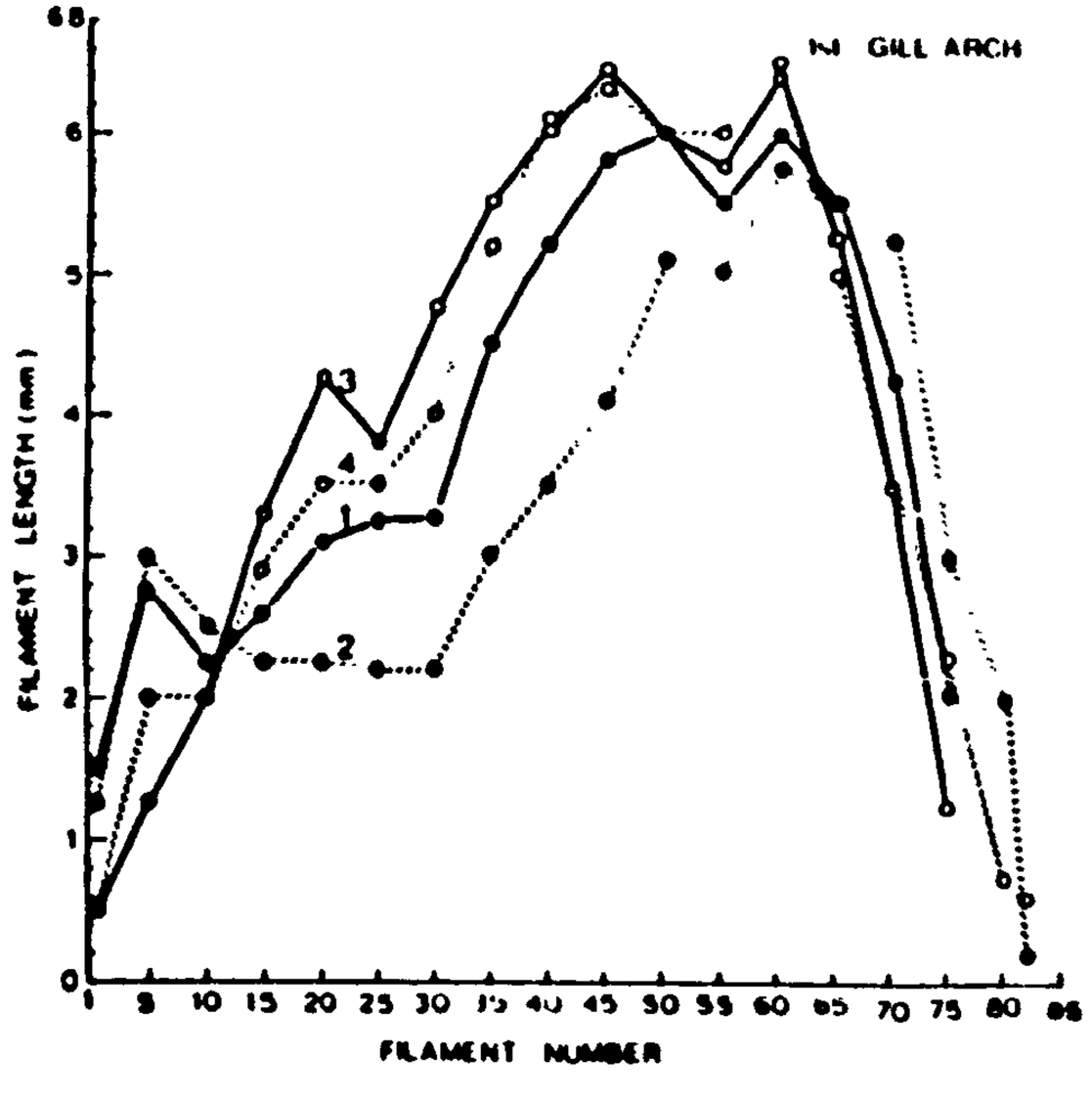
different positions along the two hemibranchs of each of the four gill arches contained in the upper and lower opercular cavities are shown in Figure 11.

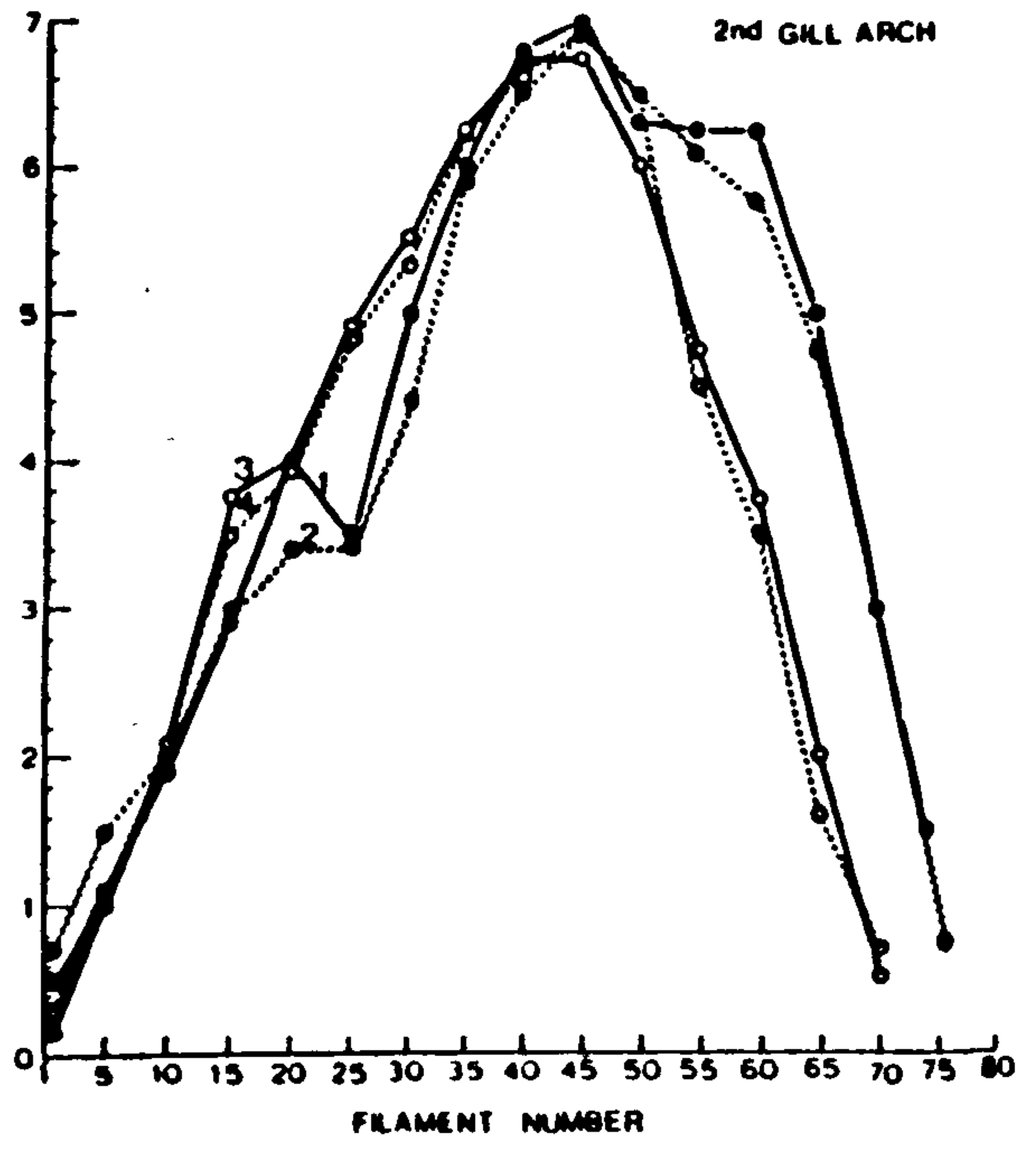
It is apparent that not only relative length of filaments of the two hemibranchs differ at different points along a given arch but also this relationship is not identical for corresponding arches of the upper and lower opercular cavities.

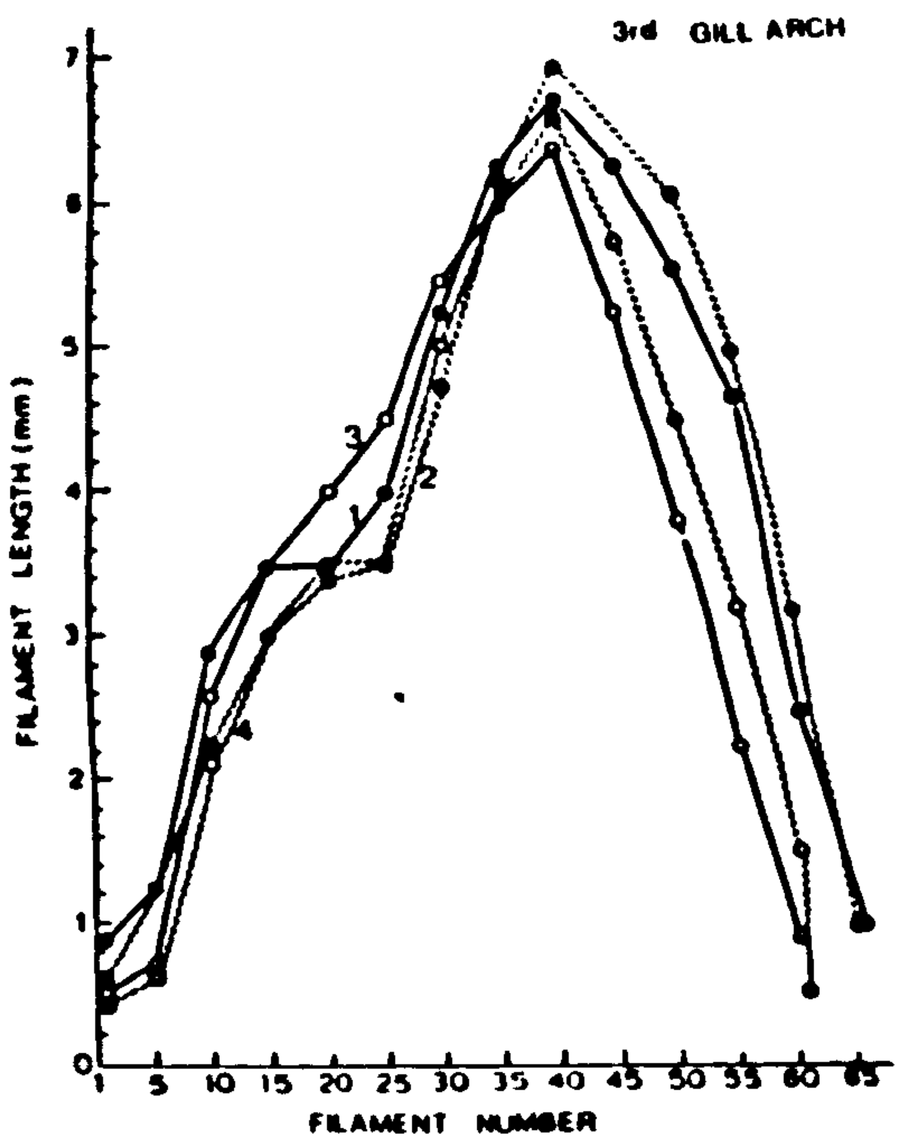
Total filament length is one of the important gill parameters which is directly proportional to total gill area. Relationships between body weight and total filament length showed high correlation for larval ($r = 0.9141$, $P < 0.001$) and adult ($r = 0.9841$, $P < 0.001$) specimens (Table 12). For a unit increase in body weight, the total filament length in larval and adult forms increased by powers of 1.0985 and 0.502 respectively (Tables 12 -14; Fig. 12).

5.3.2 RELATIONSHIP BETWEEN BODY WEIGHT AND TOTAL FILAMENT NUMBER

A detailed examination of the two hemibranchs of each of the four gill arches of the pre-metamorphic and adult stages revealed a greater number of filaments on the second gill arch (36) followed by third (34), first (28) and the fourth (20 gill arches for a fish of 0.009g body weight (Fig. 13). The same trend


1st GILL ARCH
FILAMENT LENGTH (mm)
FILAMENT NUMBER


2nd GILL ARCH
FILAMENT NUMBER


3rd GILL ARCH
FILAMENT LENGTH (mm)
FILAMENT NUMBER

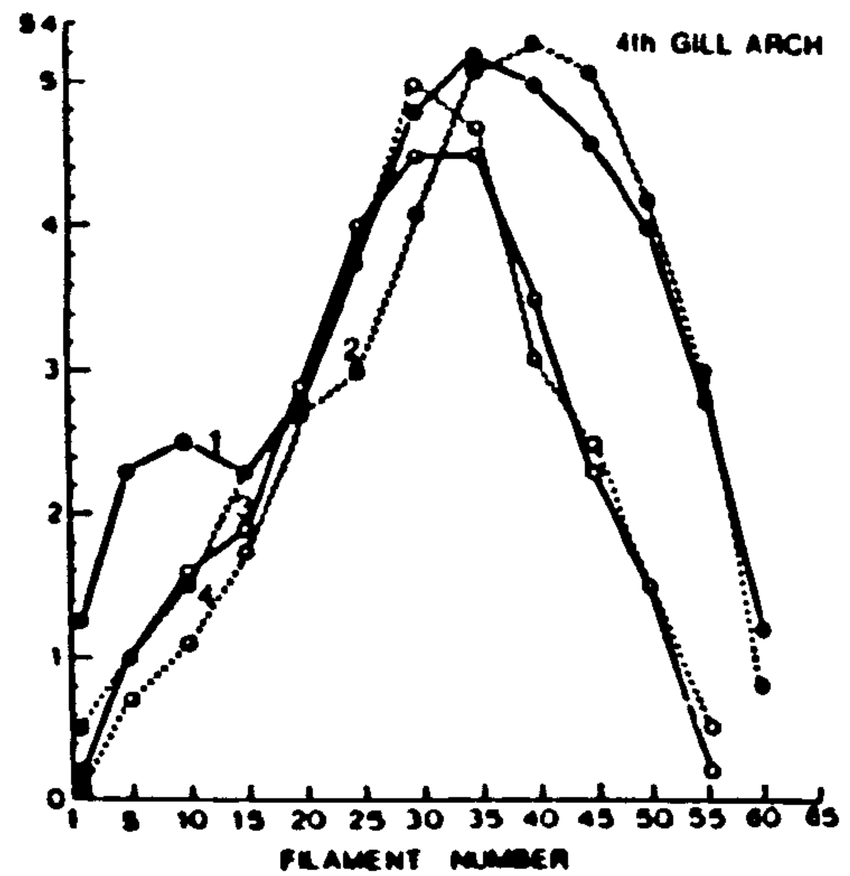

4th GILL ARCH
FILAMENT NUMBER

FIGURE 12

Bilogarithmic plot of total filament length v. body weight. (o---o, ●---●), represent regression lines before and after metamorphosis respectively

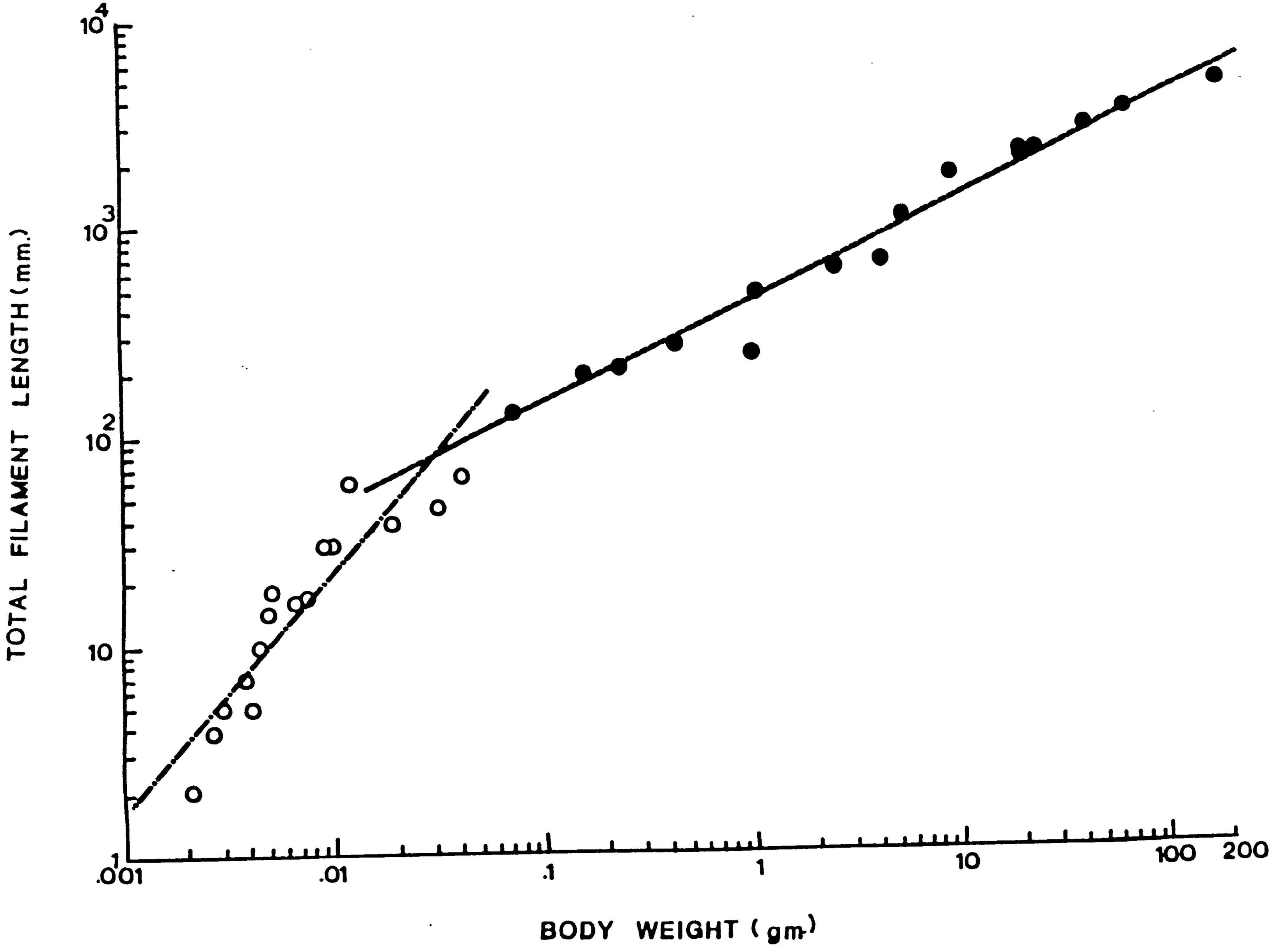
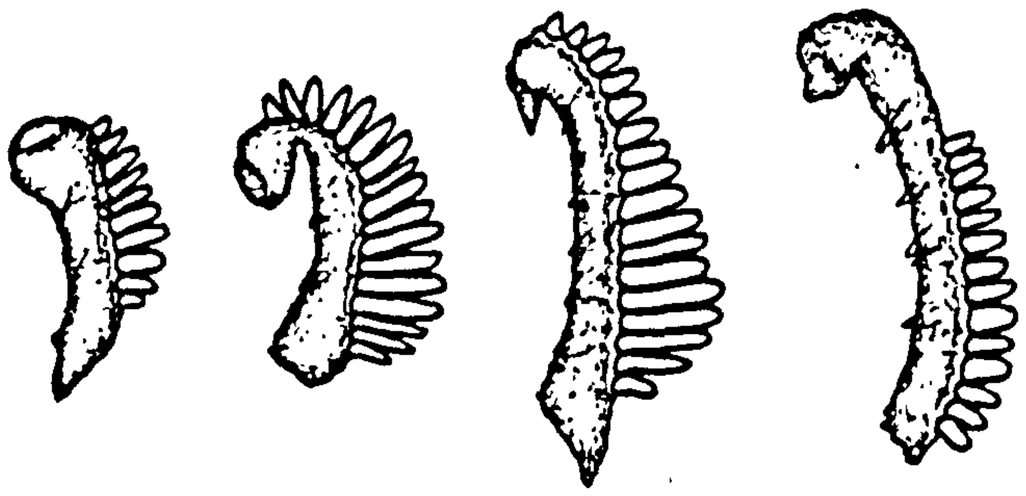
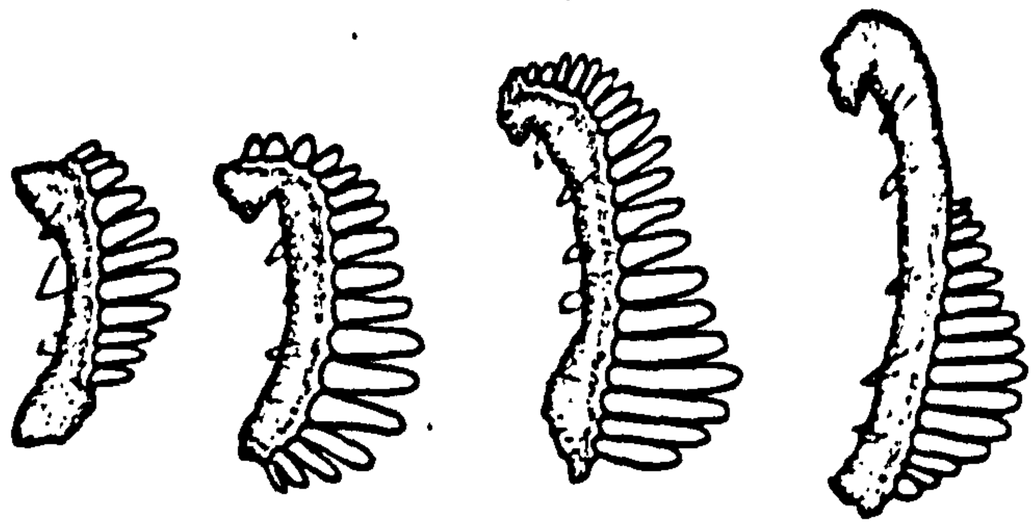

TOTAL FILAMENT LENGTH (mm.)
10^4
10^3
10^2
10
1
.001
.01
.1
1
10
100
200
BODY WEIGHT (gm)

FIGURE 13

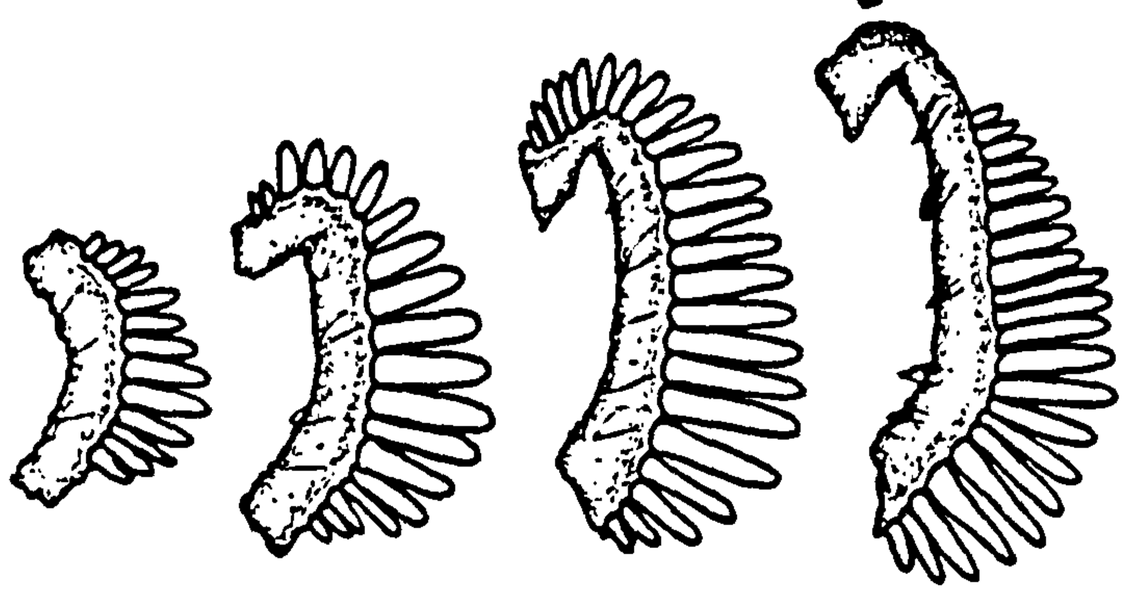
Diagrams showing the number of gill filaments at different developmental stages.



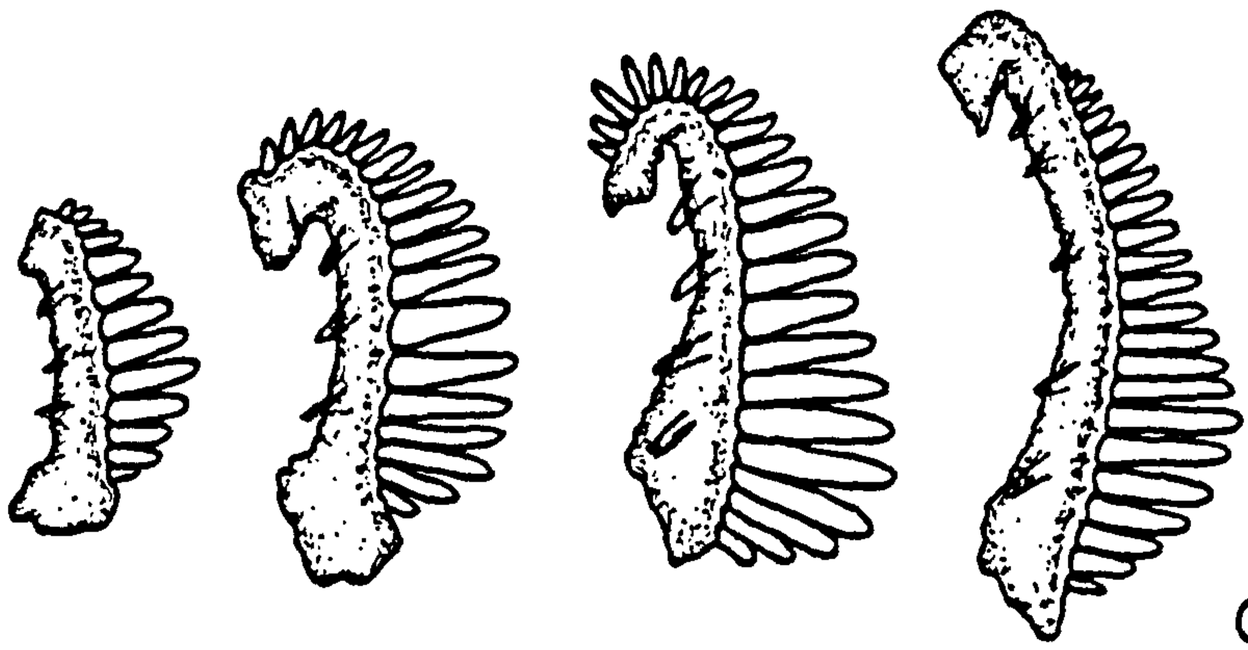
0.009 gm.
1 cm.



0.01gm.
1.1 cm.

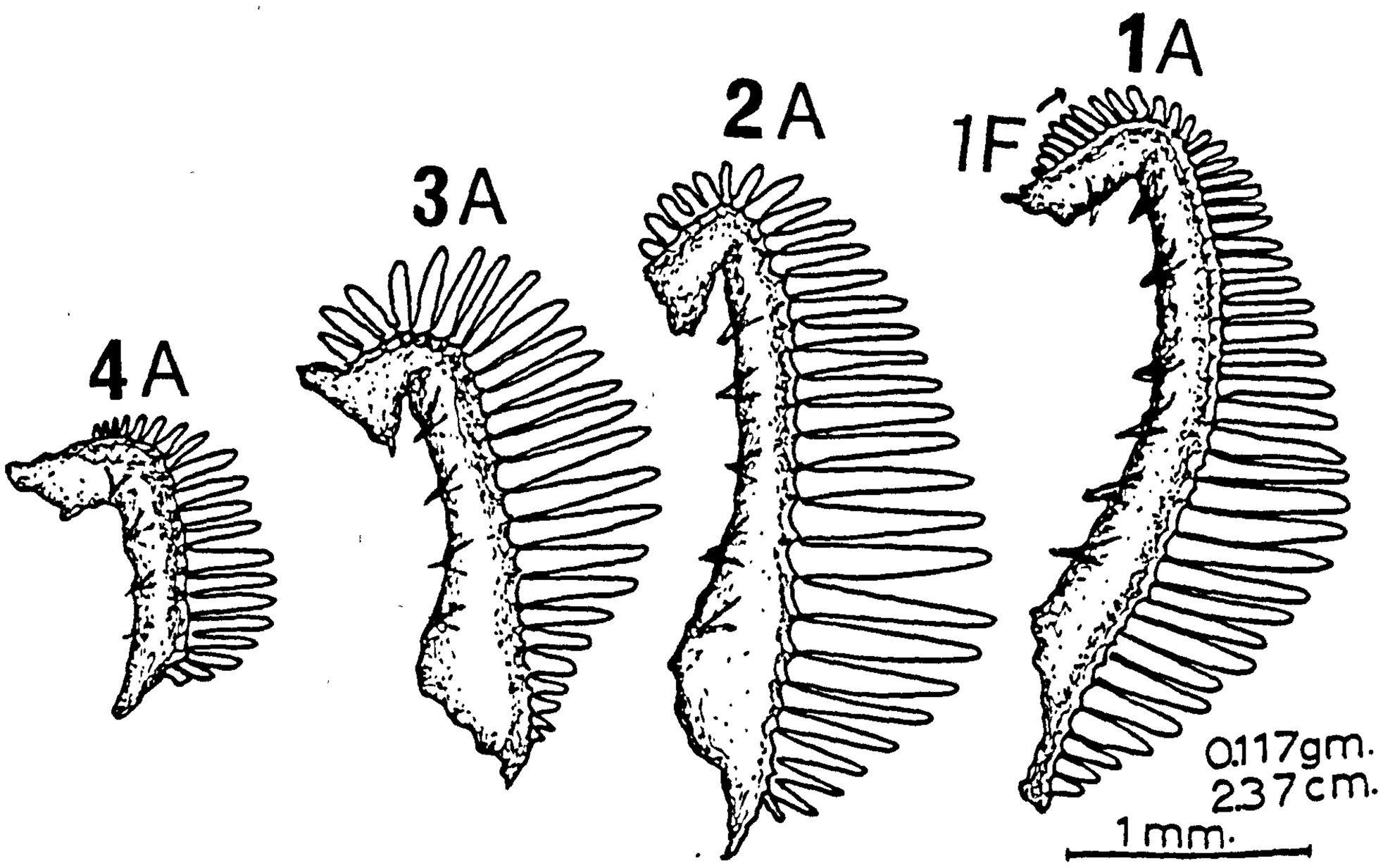


0.012gm.
1.2 cm.



0.021g
1.3 cm.

0.5mm



0.117gm.
2.37cm.

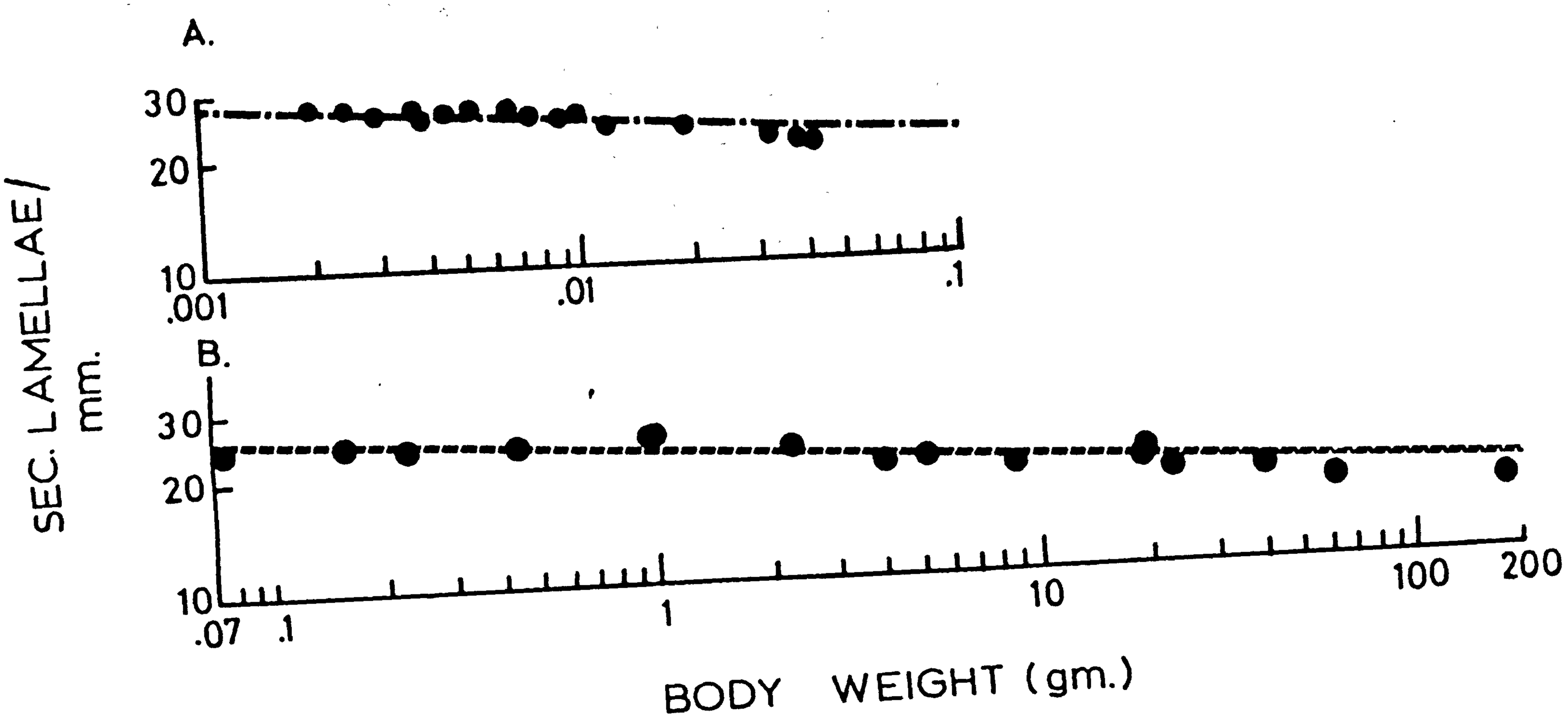
1mm.

was also observed when the fish attained the weight of 0.01g. But due to its higher growth rate, the first gill arch takes the second position with 42 filaments, followed by the third (38) and the fourth (26) gill arches in a fish of 0.012g body weight. In this weight group also the second gill arch showed a higher filament number (48). The same trend was retained in a fish of 0.021g body weight. However, for the fish of 0.117g, the highest number of filaments was recorded for the first gill arch (88), followed by the second (60), third (50), and the fourth (40) gill arches (Fig. 13) and this trend was retained for all adult fish.

Analyses of body weight and total filament number of all the arches combined together showed positive correlation for pre-metamorphic ($r = 0.9824$, $P < 0.001$) and adult stages ($r = 0.9400$, $P < 0.001$). When the data for the two variables were plotted on log-log co-ordinates, they gave straight lines with slopes of 0.2346 and 0.0857 for pre-metamorphic and adult stages respectively (Table 12).

5.3.3 RELATIONSHIP BETWEEN BODY WEIGHT AND SECONDARY LAMELLAE/MM ON BOTH SIDES OF A FILAMENT

Results of the regression analyses for frequency of secondary lamellae and body weight are presented in Tables 12-14 and shown in Figure 14 . Slopes for


A.
30
20
10
.001
.01
.1
B.
30
20
10
.07
.1
1
10
100
200
SEC. LAMELLAE/ mm.
BODY WEIGHT (gm.)

the larval and adult specimens were -0.0522 and -0.0641 respectively. Body weight and the secondary lamellae per mm showed negative correlations for larval ($r = -0.8761$; $P < 0.001$) and adult ($r = -0.9218$; $P < 0.001$) specimens (Table 12).

5.3.4 RELATIONSHIP BETWEEN BODY WEIGHT AND AVERAGE BILATERAL SURFACE AREA OF A SECONDARY LAMELLAE

The detailed measurements of the area of secondary lamellae from base, middle and tip of every filament sampled from different points on the two (anterior and posterior) hemibranchs revealed variations in their profile and dimensions (Figure 15). Furthermore, it is interesting to note that the profile and dimensions of the secondary lamellae change with increase in the body weight of the fish (Figure 16). Secondary lamellae from the middle region of a filament has the largest bilateral area because of its greater length and height in comparison to the base and tip regions (Figures 11, 15). Dimensions of individual secondary lamellae from different regions of the hemibranch are related directly to the length of the filaments (Figure 15).

In general, the larger filaments on a given hemibranch have secondary lamellae whose dimensions (length, height and thickness) are larger than the corresponding dimensions of lamellae on the shorter filaments. Thus

FIGURE 15

Diagrams showing variations in the profile and dimensions of individual secondary lamellae obtained from base (B), middle (M), and tip (T) of every 5th. filament of the anterior (A), and posterior (P) hemibranchs of the first gill arch of the upper opercular cavity (22.5g)

A - Length and height (mm)

B - Area of an individual secondary lamella (mm^2)

A

P

1 5 10 15 20 25 30 35 40 45 50 55 60 65 69

B.

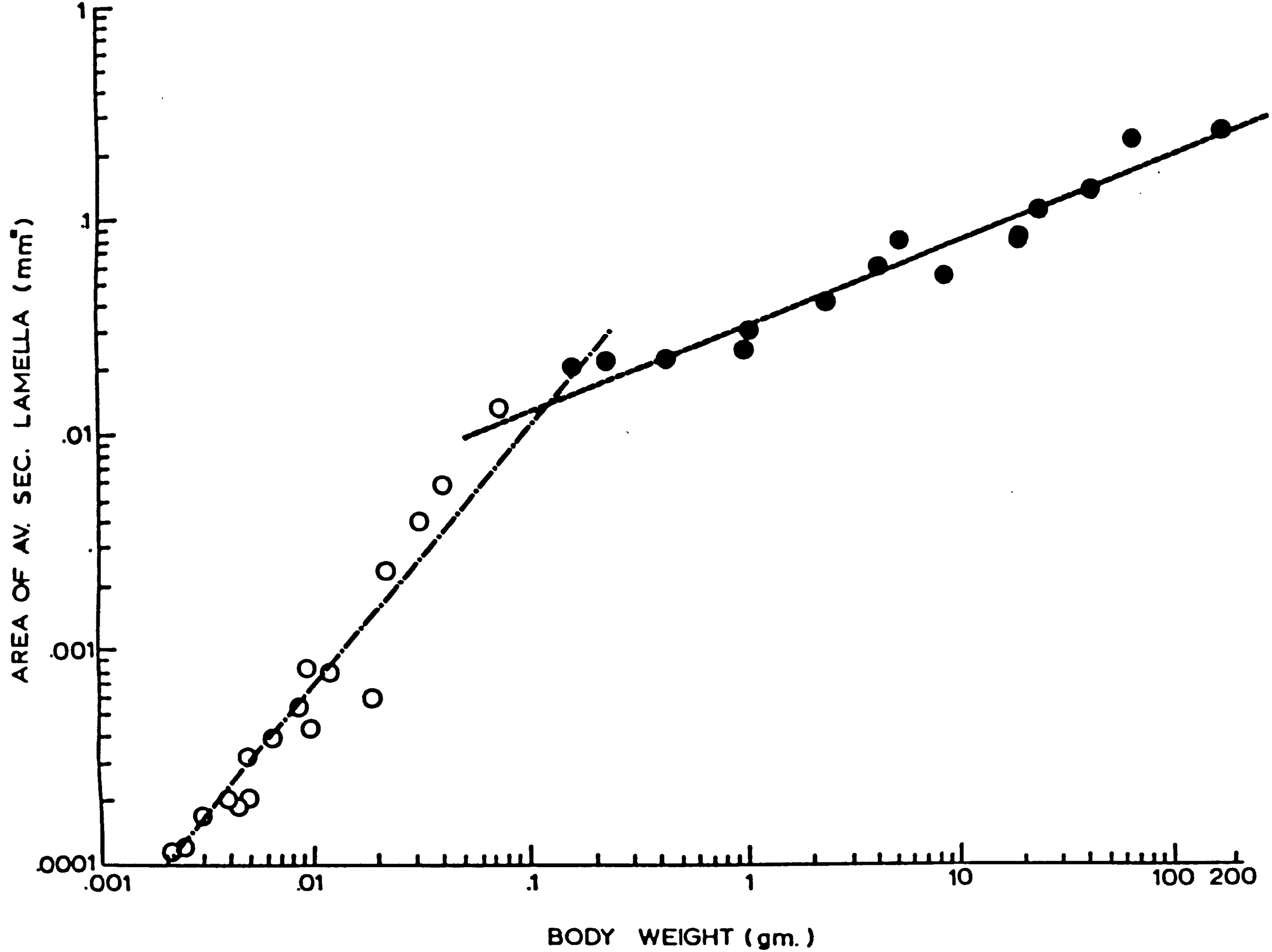
M.

T.

1 mm.

FIGURE 16

Bilogarithmic plot between area of average secondary lamellae v. body weight


AREA OF AV. SEC. LAMELLA (mm²)
1
.1
.01
.001
.0001
.001
.01
.1
1
10
100 200
BODY WEIGHT (gm.)

secondary lamellae from the middle of filaments 45-50 are larger than those from the middle region of filaments 5-10 or 60-65.

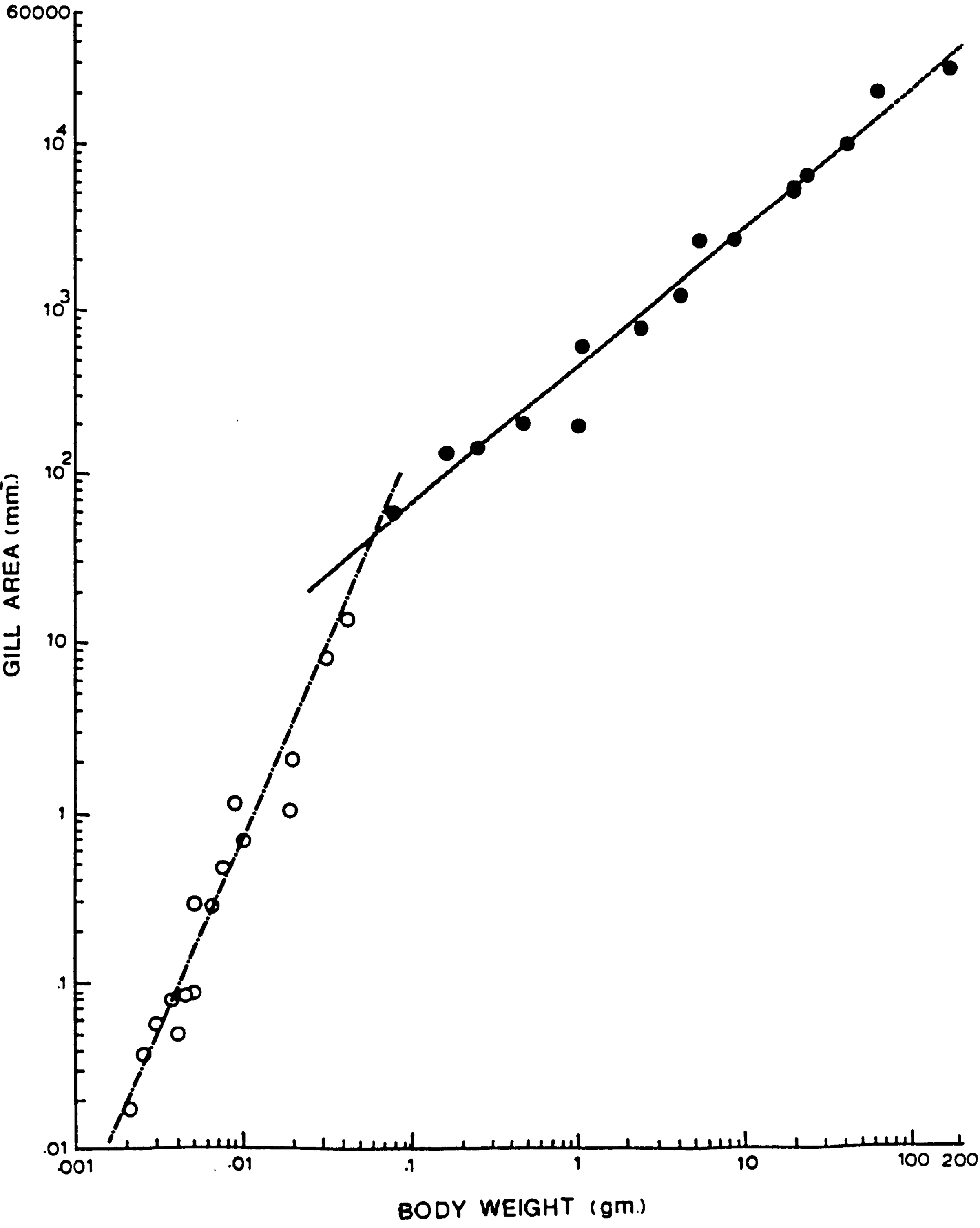
When the data for body weight and average bilateral surface area was plotted on log-log co-ordinates, they gave straight lines with slopes of 1.1832 and 0.3859 respectively for pre-metamorphic and adult stages (Figure 16). Significant correlations were obtained for both pre-metamorphic ($r = 0.9242$; $P < 0.001$) and adult ($r = 0.9734$; $P < 0.001$) stages (Table 12).

5.3.5 RELATIONSHIP BETWEEN BODY WEIGHT AND TOTAL GILL SURFACE AREA

When the gill parameters (total filament length, weighted frequency of secondary lamellae of both sides and the weighted lamellar area) are multiplied together, total gill surface area is obtained. The total gill area increased with increase in body weight. When the data for total gill surface area for larvae and adult were analysed separately, positive correlation was found with body weight for each set of measurements (Table 12). With unit increase in body weight, external surface area of the whole fish increased by powers of 2.2128 and 0.824 for pre-metamorphic and adult stages respectively (Figure 17).

FIGURE 17

Bilogarithmic plot between total gill surface area v. body weight.

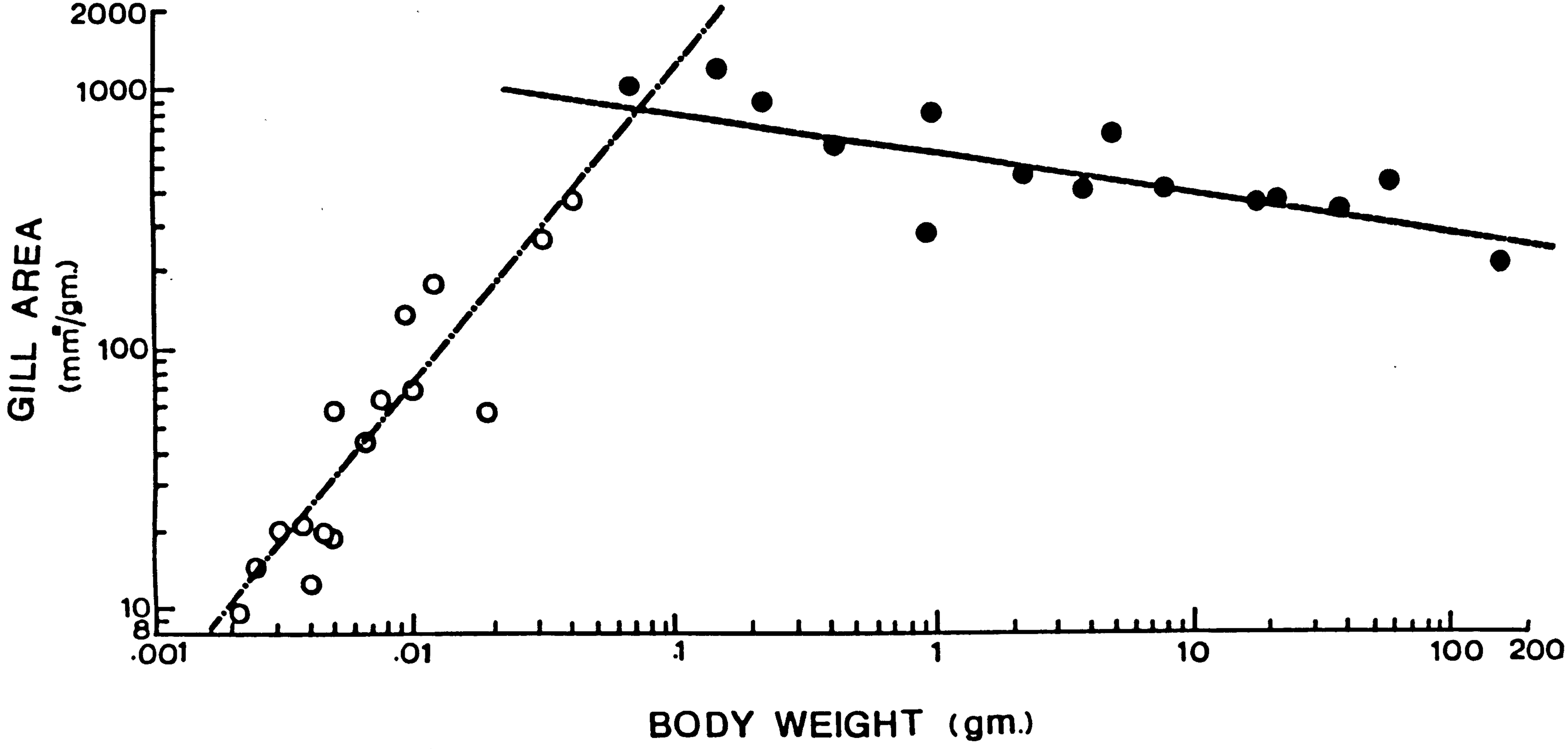

60000
10^4
10^3
10^2
10
1
.1
.01
GILL AREA (mm^2.)
.001
.01
.1
1
10
100 200
BODY WEIGHT (gm.)

5.3.6 RELATIONSHIP BETWEEN BODY WEIGHT AND WEIGHT SPECIFIC GILL AREA

Regression analysis of the data on weight specific gill area and body weight for pre-metamorphic and adult stages showed some interesting results. For pre-metamorphic stages, the slope of the regression line relating total gill area and body weight was more than 1 ($b = 2.2128$) and therefore weight specific gill area also increased with an increase in body weight. Before metamorphosis, weight specific gill area increased in proportion to $W^{1.2178}$ (Fig. 18) and showed a positive correlation ($r = 0.9104$, $P < 0.001$) (Table 12) with body weight. For adult specimens both total gill area and weight specific gill area were negatively correlated with body weight. But when these parameters were similarly plotted for adult specimens, they showed a negative correlation ($r = -0.5399$, $P < 0.001$) with a slope of -0.155 (Fig. 18). This was because the slope of the regression line relating to body weight and total gill surface area for adult stages was less than 1 ($b = 0.824$).

FIGURE 18

Bilogarithmic plot between weight specific gill area v. body weight


2000
1000
100
10
8
GILL AREA
(mm²/gm.)
.001
.01
.1
1
10
100
200
BODY WEIGHT (gm.)

5.4 DISCUSSION

The gas exchange "machinery" of Platichthys flesus is correlated with its morphological configuration and the environment in which it lives and flourishes. Adult flounders bury themselves in sand or mud, exposing only mouth, eyes and upper opercular opening. Under such circumstances, the flounders do not have the capability to ventilate their gills like round fish. In normal circumstances, the water ventilates equally the four pairs of gills located in the upper and lower opercular cavities. The water current from the lower opercular cavity appears to be directed dorsally via a communicating channel and expelled through a narrow opening in the upper side (Yazdani and Alexander,1967;Yazdani,1976). If these fish 'wish' to expel water from the lower opercular opening, they will have to apply some pressure to percolate water through sand or mud. This process is energy consuming. The lower opercular opening is only used when the fish are excited or want to remove sediments accumulated on the gills of the lower cavity (Douglas and Lanzing, 1981), or when the fish is free-swimming.

The detailed embryological and morphological studies of P. flesus reveal that in pre-metamorphic stages both opercular cavities are symmetrical. However, in adult specimens, the upper cavity is larger than the lower. The lower cavity supports the weight of

the fish in addition to a column of water over it. The lower cavity is flatter and smaller than the upper which is freely used for ventilation of the gills. The upper cavity is more convex and probably accommodates a larger volume of water than the lower cavity. As far as the explanation of this asymmetry is concerned, it may be that the problem of accommodating the extra volume of water received from the lower chamber, in addition to that received directly from the mouth, has stimulated the upper chamber to develop more rapidly than its lower counterpart. Consequently, the gills of the upper chamber are also better developed than those in the lower chamber and may play a more dominant part in gas exchange.

From the present embryological and morphometric studies of the gills in pre-metamorphic and adult stages of Platichthys flesus, it is apparent that during development the filaments first appear on the second gill arch and then on the third, first and fourth gill arches respectively. However, other fish species show different gill development patterns. In most of the fish such as bass, Micropterus dolomieu (Price, 1931), trout, Salmo gairdneri (Morgan, 1971) and plaice, Pleuronectes platessa (de Silva, 1974), the filaments first appear on the first gill arch, followed by those on the second, third and the fourth gill arches respectively. In herring, Clupea harenqus,

however, filaments are first observed on the third gill arch, followed by the fourth, second and the first gill arches respectively (de Silva, 1974). However, in adult flounders, the first gill arch has the highest number of filaments, followed by the second, third and fourth gill arches. This change in the trend may be due to a higher growth rate of filaments on the first gill arch than on the other three gill arches. The relationship between body weight and total number of filaments on all the arches together for pre-metamorphic and adult stages of flounders indicate different slope values. In the pre-metamorphic stages, the filament number increases by a power of 0.2346 with unit increase in body weight as against 0.0850 for adults. This finding indicates that the filament number increases more rapidly in larval forms than in adult forms as in other fish species.

5.4.1 FILAMENT LENGTH

From the detailed measurements of filament length at different points along the two hemibranchs of each of the four pairs of gill arches, it is clear that there is not only a wide difference in the relative size of filaments at different positions along the arches, but also that this relationship varies in different arches sampled from the upper and lower opercular cavities. These findings corroborate

the previous findings on Opsanus tau (Hughes, 1972), Mola mola (Adeney and Hughes, 1977), Pacific deep-sea fish (Hughes and Iwai, 1978) and Latimeria chalumnae (Hughes, 1980). Such an arrangement of gill filaments on the two hemibranchs of each of the four pairs of gill arches probably help in the formation and accommodation of the complete gill sieve required for efficient gas exchange in the branchial cavity. Asymmetry of gills from the upper and lower opercular cavities is a new finding and can be explained in the light of different growth patterns of the two cavities in accordance with the habit and habitat of the flounder.

Regression analysis between body weight and total filament length indicates different growth patterns for pre-metamorphic and adult stages of P. flesus. In pre-metamorphic stages, the total filament length increases more quickly (b = 1.0985) than in adult stages (b = 0.502). This ensures a greater rate of increase in gill surface during growth and is associated with the greater activity of these larval stages and consequently a higher oxygen transfer across the gills of these fish which will be aided by the increase in total gill area.

5.4.2 SECONDARY LAMELLAE

Secondary lamellae are arranged alternately on both surfaces of the gill filaments. The shape of

the secondary lamellae varies in different fish species (Byczkowska-Smyk, 1957; Hughes, 1970) but in general it is triangular, with its wider part displaced towards the efferent filament artery and the narrow part towards the afferent filament artery.

From morphometric studies of gill dimensions in pre- and post-metamorphic stages, it is apparent that with an increase in body weight the secondary lamellae not only show variations in profile but also in their frequency and dimensions.

Variations in lamellae can also be recorded when they are sampled from different parts of the same filament. The difference is more apparent in adult specimens than in pre-metamorphic stages. Because of variations in the frequency and dimensions of the secondary lamellae, the greatest source of error is the selection of three lamellae from the sampled filament. Furthermore, errors can also be introduced by the selection of a single arch for detailed measurements(Table 15). Quantitative estimates of errors which arise when only a single arch of P. flesus is sampled for measurement of total area support this viewpoint. Therefore, to minimise errors in the present study comparable regions were sampled in all specimens for measuring frequency and average bilateral area of the secondary lamellae.

Table 15. Summary of measurements showing variations in total gill area of an individual *Platichthys flesus* (S. Length = 8 cm, Weight = 5.1g) using various combinations of samplings.

Gill parameters	Av. Bil. Surf. Area (mm^2)	Av. No. of sec. lam./mm both sides of fil.	Gill area(mm^2)/ filament	Total fil. length (mm)	Total gill area (mm^2)
1st. gill arch	0.0666	42.7030	2.8440	351.7900	1000.4908
2nd. gill arch	0.1171	42.4720	4.9735	268.1860	1333.8231
3rd. gill arch	0.0826	43.2050	3.5687	237.1600	846.3607
4th. gill arch	0.0594	44.1200	2.6207	150.5200	394.4720
Total gill arches	0.0814	43.1250	3.5104	1007.6560	3575.1345
1st. gill arch sampled for Av. Bil. surf.area & Av. No. sec. lam./mm	0.0666	42.7030	2.8446	1007.6560	2865.7737
2nd. gill arch sampled for Av. Bil. surf. area & Av. No. sec. lam./mm	0.1171	42.4720	4.9735	1007.6560	5011.5771
3dr. gill arch sampled for Av. Bil. surf. area & Av. No. sec. lam./mm	0.0826	43.2050	3.5687	1007.6560	3596.0220
4th. gill arch sampled for Av. Bil. surf. area & Av. No. sec. lam./mm	0.0594	44.1200	2.6207	1007.6560	2640.7641

5.4.3 FREQUENCY OF SECONDARY LAMELLAE PER UNIT LENGTH OF FILAMENT

Frequency of secondary lamellae per unit length of filament is one of the important parameters which influences the total gill area. In most fish, the frequency of secondary lamellae per unit filament length decreases with increase in body weight (Table 16). However, in plaice, Pleuronectes platessa and herring, Clupea harengus, the number of secondary lamellae per unit filament length increases with increase in body weight during development (de Silva, 1974).

In the present study, regression lines of negative slope indicate a decrease in secondary lamellar frequency with unit increase in body weight throughout the whole life cycle. In pre-metamorphic and adult stages, the secondary lamellae per unit filament length decreases by a power of -0.0522 and -0.0641 respectively. These coefficients lie between the various slope values reported for other fish species. A decrease in the frequency of secondary lamellae per unit length of filament increases the distance between adjacent lamellae. Because of the greater interlamellar distances a larger quantity of water passes between the secondary lamellae without being involved in gaseous exchange and this may be regarded as a physiological dead space (Hughes, 1966).

Consequently, the resistance to gas transfer between the water and blood will be increased. Higher values for secondary lamellae/mm for pre-metamorphic stages (56.9) indicate a gas exchange machinery in comparison with that of adult specimens (45.8). Values of secondary lamellae per mm for flounders fall between the values reported for the very active tunny and sluggish fish like Callionymus lyra, Opsanus tau and Latimeria chalumnae (Table 16). This finding suggests sluggish activity for flounders.

5.4.4 AVERAGE BILATERAL SURFACE AREA OF A SECONDARY LAMELLA

Regression analysis of body weight and average bi-lateral surface area of a secondary lamella reveals that in pre-metamorphic stages the lamellar area increases more rapidly (b = 1.1832) than in adult (b = 0.385) specimens.

Variations in these exponent values may be correlated with the differences in the metabolic activity of pre-metamorphic and adult stages of P. flesus. As has been reported for other fish species (Muir and Hughes, 1969; Hughes et al, 1974; Hakim et al, 1978), the sum of the slope values for total filament length, bilateral surface area of an average secondary lamella and the number of secondary lamellae/mm gave the values 2.2297 and 0.8239 respectively for pre-metamorphic and adult stages (Table 16). These

Table 16. Regression coefficient (b) for total filament length, secondary lamellae/mm, bilateral surface area of an average secondary lamella and their sum in different fish species for comparison with slope (b) for the total gill area in relation to body weight.

Fish species	Slope (b) total filament length (1)	Slope (b) secondary lamellae/ mm. (2)	Slope (b) bilateral surface area (3)	Sum of cols. 1,2 and 3 (4)	Slope (b) total gill area (5)	References
Coryphaena hippurus	0.4310	-0.0360	0.3270	0.7720	0.7130	Hughes (1970a)
Scomber scombrus	0.4110	-0.0234	0.5560	0.9904	0.9970	Hughes (1970b)
Scyliorhinus canicula	0.3510	-0.0710	0.6840	0.9640	0.9610	
Tinca tinca	0.3190	-0.0160	0.1860	0.5210	0.5220	
Opsanus tau	0.4850	-0.0750	0.3720	0.7820	0.7900	Hughes & Gray (1972)
Platichthys flesus (*)	1.0985	-0.0520	1.1832	2.2297	2.2128	Present study
Platichthys flesus (")	0.5020	-0.0641	0.3850	0.8239	0.8240	Present study

(*) Pre-metamorphic stages

(") Post-metamorphic stages

values approach the slope values obtained for the regression line of the total gill surface area in relation to body weight for pre-metamorphic (b = 2.2128) and adult (b = 0.8240) respectively. These findings support the use of a logarithmic transformation for data computation.

5.4.5 TOTAL GILL AREA RELATING TO BODY WEIGHT

The slope of the regression line relating total gill area and body weight was first thought to be close to the value reported for the general aerobic metabolism/weight relationship found in fish (Muir and Hughes, 1969). However, more detailed analyses have indicated that these relationships may vary from 0.5 to 1.28 in different fish species. In the present study, the coefficient (0.824) relating gill area and body weight for adult specimens falls within the range of values so far reported for other fish species (Table 17), but the b-value obtained for the pre-metamorphic stages is high (b = 2.2128). In closely related studies on herring, Clupea harengus and plaice, Pleuronectes platessa, the slope of the gill area : body weight relationship is also greater before than after metamorphosis. In herring, it decreases from values of 3.36 to 0.79, while in plaice it goes down from 1.59 to 0.85 (de Silva, 1974).

The higher value for pre-metamorphic stages in the

Table 17. Summary of exponent (b) values for gill area $A = aW^b$ reported for various fish species

Specimens	b-value	Authors
Platichthys flesus (flounder)	0.824	Present study
Barracuda	1.281	Hughes, 1980
Scomber scombrus (mackerel)	0.997	Hughes, 1970b
Scyliorhinus canicula (dogfish)	0.961	Hughes, 1970b
Salmo gairdneri (trout)	0.95	Morgan, 1971
Thunnus albacares (yellow tuna)	0.875	Muir & Hughes, 1969
Thunnus thynnus (bluefin tuna)		
Blennius pholis (shanny)	0.850	Milton, 1971
Katsuwonus pelamis (skipjack tuna)	0.850	Muir & Hughes, 1969
Pleuronectes platessa (plaice)	0.850	de Silva, 1974
Gray's intermediates	0.820	Ursin, 1967
Opsanus tau (toadfish)	0.790	Hughes, 1970a and Hughes & Gray, 1972
Micropterus dolomieu (small-mouthed bass)	0.780	Price, 1931
Clupea harengus (herring)	0.780	de Silva, 1974
Coryphaena hippurus (dolphin fish)	0.710	Hughes, 1970a
Tinca tinca (tench) (1970)	0.698	Hughes, 1970b
Latimeria chalumnae	0.614	Hughes, 1979
Tinca tinca (1969)	0.552	Hughes, 1970b

present study is associated with high metabolic demand during rapid growth. After metamorphosis, the metabolic demand and growth is reduced. Development of the various parameters affecting total gill area slows down and this results in a comparatively low value for the slope of the regression line relating gill area and body weight for adults. The slope value obtained for adult specimens is lower than the values reported for very active fish such as Thunnus albacares, T. thynnus, Katsuwonus pelamis (Muir and Hughes, 1969), Scomber scombrus and Scyliorhinus canicula (Hughes, 1970b) and Pleuronectes platessa (de Silva, 1974) but higher than those reported for Micropterus dolomieu (Price, 1931) and Clupea harengus (de Silva, 1974) (Table 17).

The A_{200} (gill surface of a standard 200g fish mm^2/g) value for P. flesus (253.12) is lower than the values reported for very active fish like Katsuwonus pelamis (2444), Thunnus albacares (2040), Thunnus thynnus (1446), Coryphaena hippurus (1382), but higher than the values reported for more sluggish fish like Opsanus tau (184) and close to values reported for closely related members of the family Pleuronectidae (De Jagger and Dekkers, 1975). Therefore, from a comparison, the A_{200} values for flounders is closer to the values reported for sluggish fish than those for active fish.

5.4.6. GILL AREA/G BODY WEIGHT

From the previous discussion, it is clear that in adult flounders the total gill area increases with weight by a power less than 1 (b = 0.824). Therefore, weight specific (area/gram) gill area decreases by a power of -0.155 with weight. This negative slope value for P. flesus suggests a decrease in activity with increase in body weight.

Detailed statistical analyses of the various gill parameters with body weight suggests a two-component curve for the regression line. This may be meaningful in relation to the habit and activity of P. flesus. The greatest slope value was obtained for fish below 0.05g. This suggests that pre-metamorphic stages are more active than adult stages. The presence of a diphasic relationship between gill development and body size is of particular interest in these fish, and it gives a quantitative indication of differences in the mode of life and oxygen requirements of the pre-metamorphic and adult stages.

Chapter 6: EMBRYONIC DEVELOPMENT OF FLOUNDER, PLATICHTHYS FLESUS (L.)

6.1 INTRODUCTION

The embryonic development of the gills has become of great interest in recent years, partly because of an interesting amount of research into fish respiration. An early studies of embryonic development of gills, in elasmobranchs was investigated by Goette (1901), and in teleost by Goette (1878), and Moroff (1902; 1904). Grodzinski (1948) studies the circulatory system in teleost development and show the principal vessels connected with the respiratory organs. Some aspects of the development of the gills and branchial vessels in trout have been discussed by Swertzoff (1923), which is contradicted by recent studies (Markiewicz, 1960; Solewski, 1949). The circulatory system in the flounder studied by Biborski (1935), who gave special attention to the main afferent and efferent vessels but not in the gills.

Recently, many studies have been carried out on gill structure in adult fish (Byczkowska-Smyk, 1961; De Silva, 1974; Hamada, 1968; Holliday & Jones, 1967; Hughes & Grimstone, 1965; Kempton, 1969; Morgan, 1971; 1974; Morgan & Tovell, 1973; Newstead, 1967; Rhodin, 1964; Wright, 1973; 1974; ...and others).

A knowledge of the morphological development of gills

is important to interpretation of physiological studies, such as that by Holeton (1971), which can in turn provide information on respiratory function in the adult. Variation in the pattern of gill blood vessels in different adult teleosts have a phylogenetic significance (Muir, 1970; Nelson, 1967; Petukat, 1965; Sewertzoff, 1923), and ontogeny of gill blood vessels can aid in such studies.

The present chapter, in addition to a study of the embryonic development of the gill, will illustrate the relationships between heart beat frequency, opercular frequency, heart mass, and length with body weight.

C H A P T E R 6

EMBRYONIC DEVELOPMENT OF FLOUNDER, *PLATICHTHYS FLESUS* (L.)

6.2 MATERIALS AND METHODS

Fertilised eggs, embryonic phases, and adults of the flounder, P. flesus, were reared from eggs in the laboratory in running sea-water at 13°C.

Specimens were fixed at various intervals for the period of pre- and post-metamorphic stages. Live specimens were examined with the help of a dissecting microscope using a similar experimental set-up to that used by Morgan (1974).

(For fixation, embedding, and sectioning see Chapter 2, Materials and Methods in this thesis).

6.3 RESULTS

6.3.1 General development of P. flesus

The one day old embryo can be recognised by the presence of the dorsal and ventral (yolk-sac) parts encased in egg membrane (Figure 19a).

The two day old embryo is recognised by the presence of a segmented body, colourless eyes, undifferentiated brain and rudimentary heart. Differentiation of notochord and spinal cord can also be recognised (Figure 19b). During the period when the mouth is closed the embryos depend mainly on yolk and cutaneous respiration.

After four days, the embryos come out of the egg membrane and float at the surface. They are transparent. The heart starts beating (84/minute). The brain is differentiated and the digestive trunk is also differentiated as a tube. Pigments were discernible on the skin of the embryos. Auditory organs also differentiate (Figure 19c). A large yolk sac is also visible. At this stage the mouth opening and jaws were not discernible.

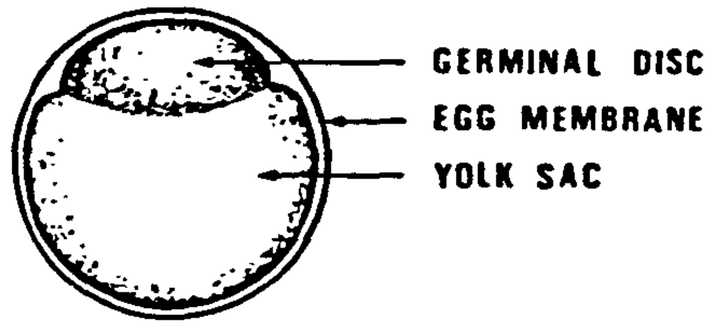
On the sixth day, the larva becomes straight and the heart was observed to beat 84-90 times/minute. Gill arches also differentiate during this period. The

FIGURE 19

Diagrams showing different developmental stages.

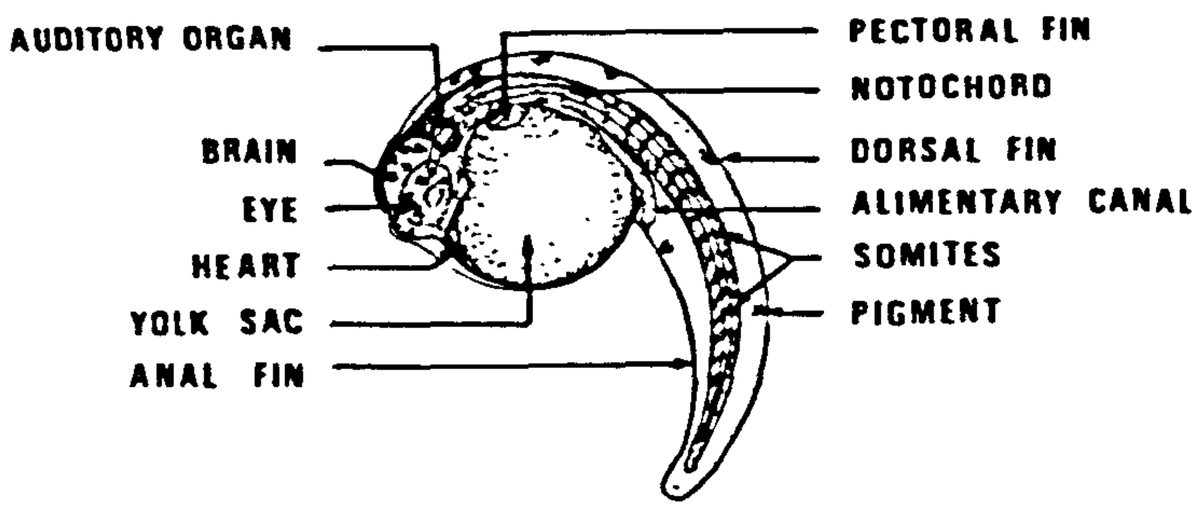
a. 1 day old
b. 2 days old
c. 4 days old
d. 6 days old
e. 9 days old
f. 11 days old
g. 44 days old
g. 120 days old

FIGURE 19

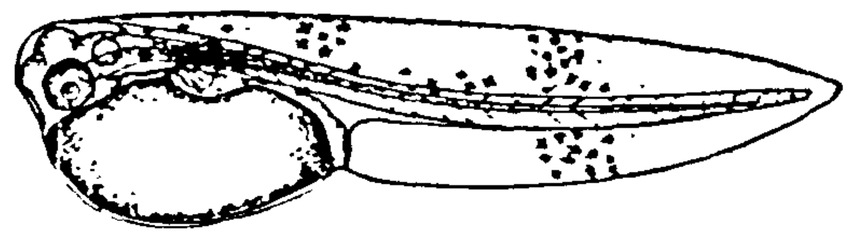


a. 1 DAY OLD

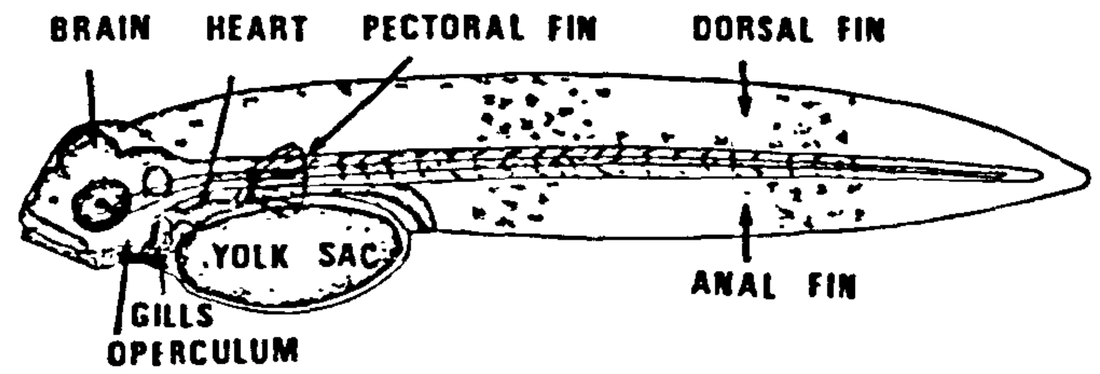
b. 2 DAYS OLD



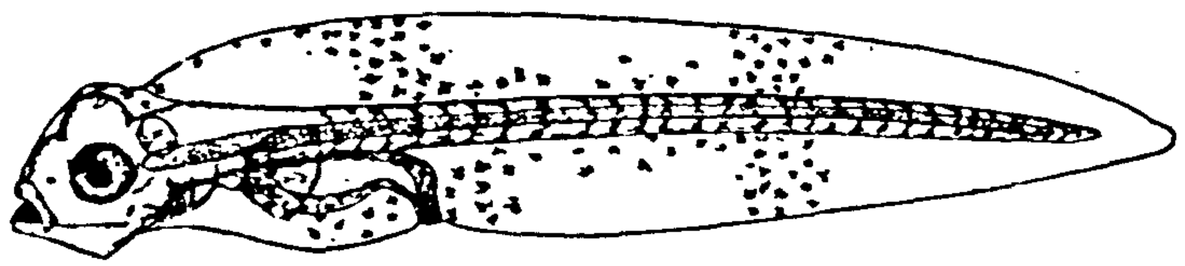
c. 4 DAYS OLD



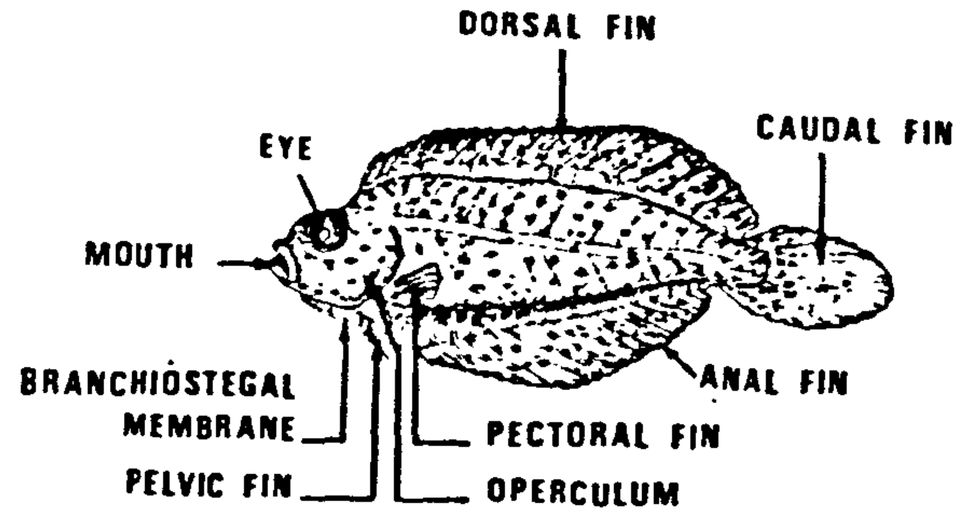
d. 6 DAYS OLD



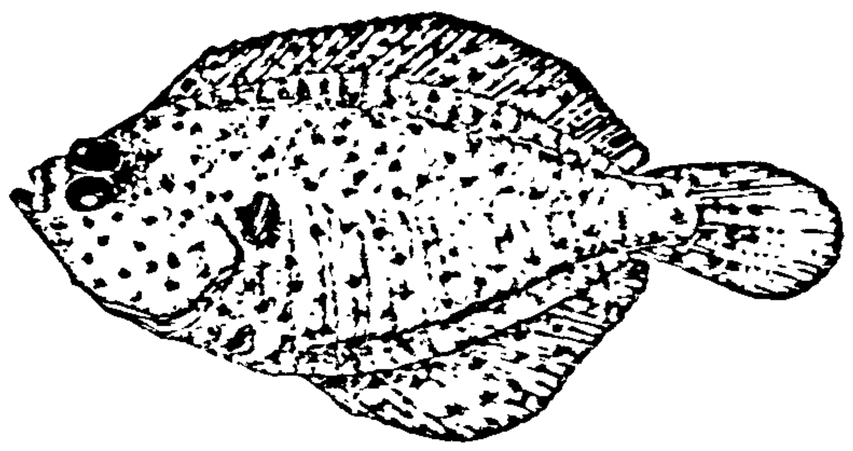
e. 9 DAYS OLD



f. 11 DAYS OLD



g. 44 DAYS OLD



h. 120 DAYS OLD

1 mm
a,b,c,d,e,f

1 mm
g,h

larvae were transparent and active (Figure 19d).

On the ninth day, the upper and lower jaws were differentiated but still the mouth remained closed. The dark eyes were placed on the antero-lateral side of the head. The development of gill filaments starts during this period. The yolk sac is very much reduced. Many chromatophores could be seen on the skin of the embryo. Because of the absence of mouth and operculum, larvae continue to depend mainly on yolk and cutaneous respiration (Figure 19e).

On the 11th day, the larvae settle to the bottom of the tank. The mouth and opercular chambers open and there was no trace of the yolk sac and feeding started. The eyes were lateral in position (Figure 19f).

The larvae feed actively on artificially-cultured food (Artemia nauplii and Rotifera), and on the 44th day they become a miniature fish. However, the eyes are still on both sides of the head. The dorsal, anal, caudal, pectoral and pelvic fins become well developed. Branchiostegal membranes could also be recognised in 44 day old larvae (Figure 19g).

On the 120th day, the fish with adult characterisations was seen. The two eyes shifted to the upper side of the head (Figure 19h).

6.3.2 Development of gills

Differentiation of gill arches starts at day six, but the gill filaments appear at 14 days. Differentiation takes place in a caudal direction from the first gill arch (Plate 16a).
On the 15th day, the second gill arch also shows the differentiation of gill filaments in the form of small buds. During this period, the endocardial tube is also recognisable (Plate 16b).
However, in the sections, the branchial and filament arteries were not discernible.

In sections of 22 day old larvae, afferent and efferent branchial arteries are well recognised. Afferent and efferent filament arteries in the gill filaments are visible. In some of the sections, afferent and efferent branchial arteries are joined together. However, differentiation of secondary lamellae was not visible. A well formed heart with auricle and ventricle could be seen in sections of this stage (Plate 16c). The connection between afferent and efferent filament arteries can also be seen in a magnified view of the gill filament

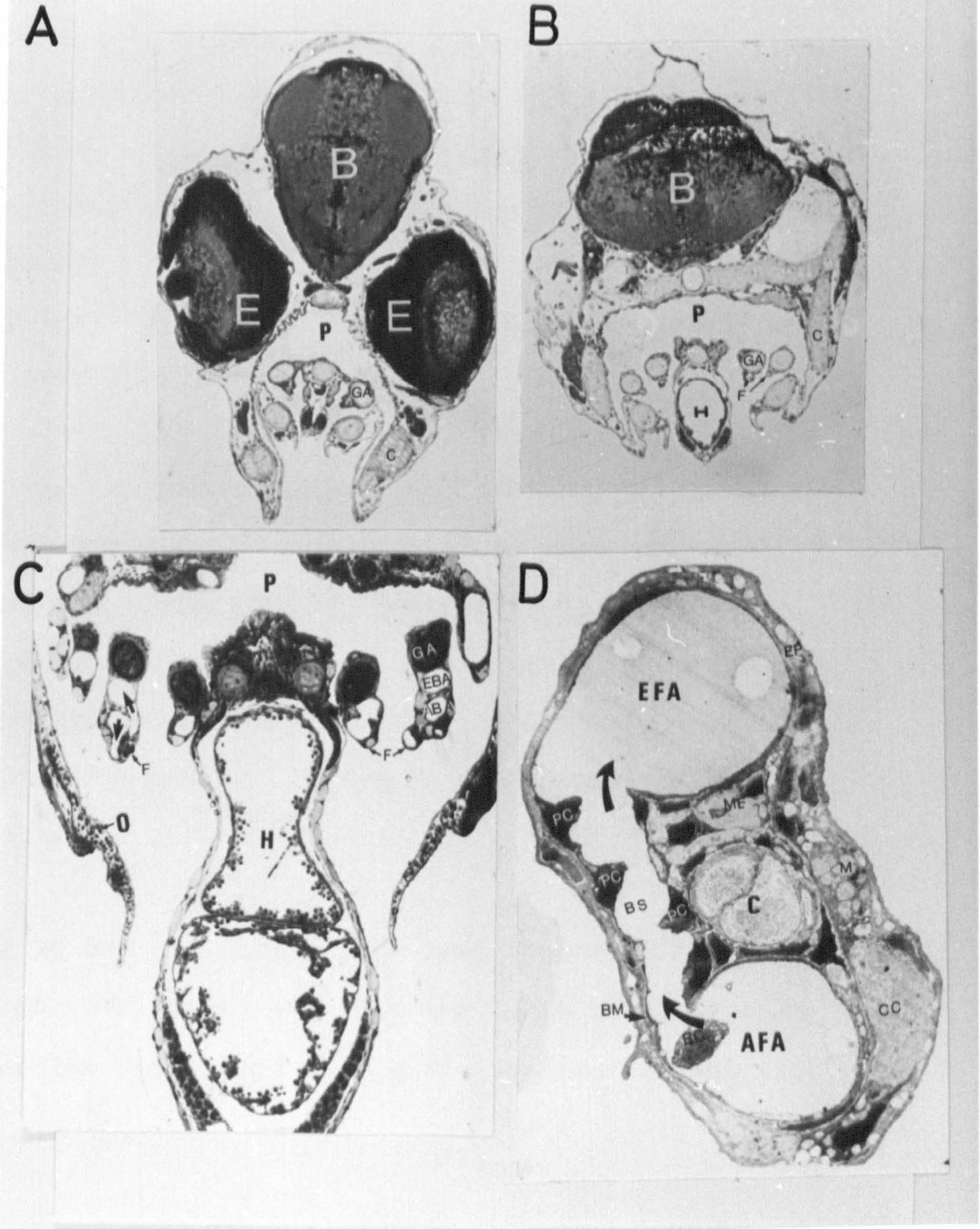
PLATE 16

a. Light micrograph of transverse section through eyes (E) and pharynx (P) of 6 days old flounder. Brain (B); cartilage (C); gill arch (GA) are also shown. (X 50)

b. Light micrograph of transverse section through head of 6 days old flounder. Cartilage (C); gill arch (GA); heart (H); pharynx (P) are also visible. (X 50)

c. Light micrograph showing transverse section heart of 22 days old flounder. Note the direction of blood flow from afferent branchial artery (ABA) to efferent branchial artery (EBA) (thick arrows). Filament (F); operculum (O); pharynx (P) ;heart (H) are also shown.
(X 50)

d. Electron micrograph of a transverse section through gill filament of 22 days old flounder. Note the direction of blood flow from afferent (AFA) to efferent filament artery (EFA) (thick arrows). Basement membrane (BM); blood cell (BC); blood space (BS); cartilage (C); chloride cell (CC); mitochondria (M); pillar cell (PC); epithelial layer (EP)
(X 1,750)


A
B
E
E
P
GA
C
B
B
P
C
GA
H
F
C
P
GA
EBA
AB
F
O
H
D
EFA
EP
PC
PC
PC
BS
ME
C
M
BM
AFA
CC

of the twenty-two day old embryo (Plate 16c). Electron micrographs of sections of developing fil filament of 22 day old larvae show the differentiation of marginal channels with endothelial lining. At the junction of the afferent filament artery and the marginal channel, a sphincter-like structure was seen through which the RBC squeezed into the marginal channel. Chloride cells and the cartilaginous gill ray were also visible in these sections (Plates 16c, 17a, 17b).

In the sections of the 35 day old embryo, moderately developed gill filaments could be seen with well formed vascular units. Secondary lamellae with blood channels separated by pillar cells were also seen in the sections (Plates 18a & 18b). The blood channels are formed by the flanges of pillar cells.

The 55 day old larvae show well organised gills, in which respiratory and vascular units of the cardio-vascular system are also well organised (Plate 18b).

In the 88 day old larvae, a well defined central venous sinus lined with endothelium was recognised (Plate 18c). In the sections, some amoebocytes could also be seen.

PLATE 17

a. Electron micrograph of section through gill filament of 22 days old flounder. Note the parts of lamellae embedded in the filament epithelia which consist mainly of chloride cell (CC). Note the abundant mitochondria (M) in chloride cell. Basement membrane (BM); endothelial cell (EN); epithelial layer (EP); white blood cell (WBC) are also shown. (X 6,800)

b. Electron micrography of section through gill filament of 22 days old flounder. Note the first marginal channel (MCH) and pillar cell (PC). Also, the undifferentiated pillar cell (UPC) at the base. Afferent filament artery (AFA); cartilage (C); blood space (BS); white blood cell (WBC) are also visible.

(X 6,800)

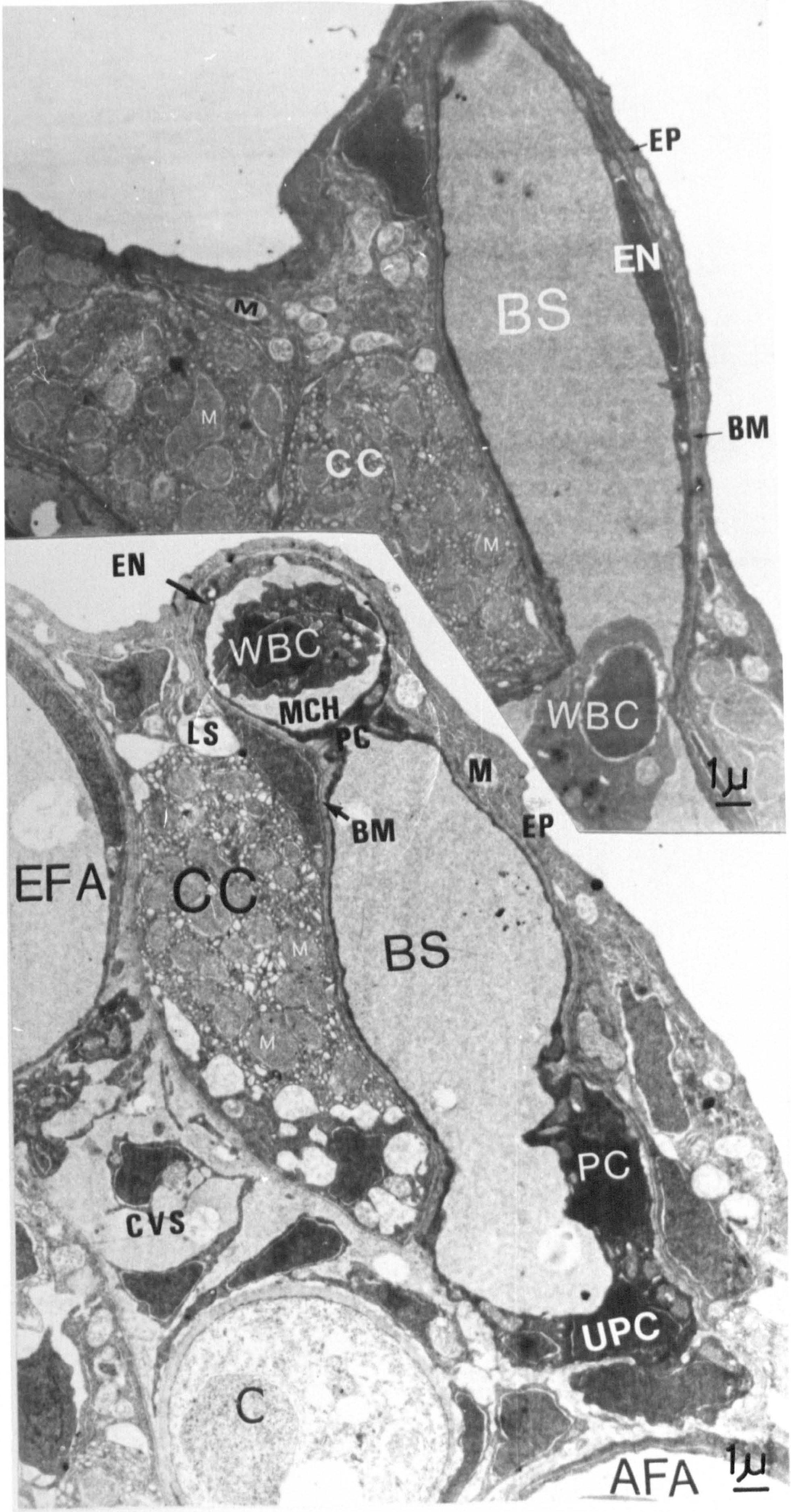

EP
EN
BS
M
BM
M
CC
M
EN
WBC
MCH
LS
PC
M
BM
EP
WBC
1µ
EFA
CC
M
M
BS
PC
CVS
UPC
C
AFA
1µ

PLATE 18

a. Light micrograph of transverse section through pharynx (P) of 35 days old flounder. Gill (G); operculum (O); and pseudobranch (PS) are also shown. (X 50)

b. Light micrograph of transverse section through head of 55 days old flounder. Afferent branchial artery (ABA); efferent branchial artery (EBA); filament (F); pillar cell (PC) are also shown. (X 50)

c. Electron micrograph of section through a gill filament showing the efferent filament artery(EFA); mesenchyme cells (ME); basement membrane (BM); and chloride cell (CC). (X 12,000)

p

PS

O

G

1mm.

EBA

ABA

PC

F

1 mm.

CC

BM

ME

EFA

1µ

6.3.3 Heart beat frequency in relation to body weight

In lower weight groups (0.001-0.0906g) of P. flesus, the heart beat frequency per minute ranged from 62 - 156 min^{-1}. When the data of heart beat frequency were plotted on log-log co-ordinates they gave a straight line with a slope of -0.2178 Figure 20). The two variables showed a negative correlation ($r = 0.9069$; $P < 0.001$).

6.3.4 Opercular frequency in relation to body weight

In the lower weight groups of P. flesus, the opercular frequency per minute ranged from 48 - 150 min^{-1} in the weight range of 0.0014 to 0.096g (Table 18).

When the data of opercular frequency were plotted on log-log co-ordinates they gave a straight line with a slope of -0.1146 (Figure 20).

6.3.5 Length-weight relationship

The length-weight relationship of young and adult P. flesus suggests a two component curve (Tables 19,20,Fig. 21) The slope of the regression line relating body length and body weight for young stages was less (2.3755) than that obtained for adult stages (2.9636). The correlation coefficients between two parameters for young ($r = 0.9528$) and adult ($r = 0.9969$) stages show high correlations.

FIGURE 20

a. Bilogarithmic plot between heart beat frequency v. body weight.

b. Bilogarithmic plot between opercular frequency v. body weight.

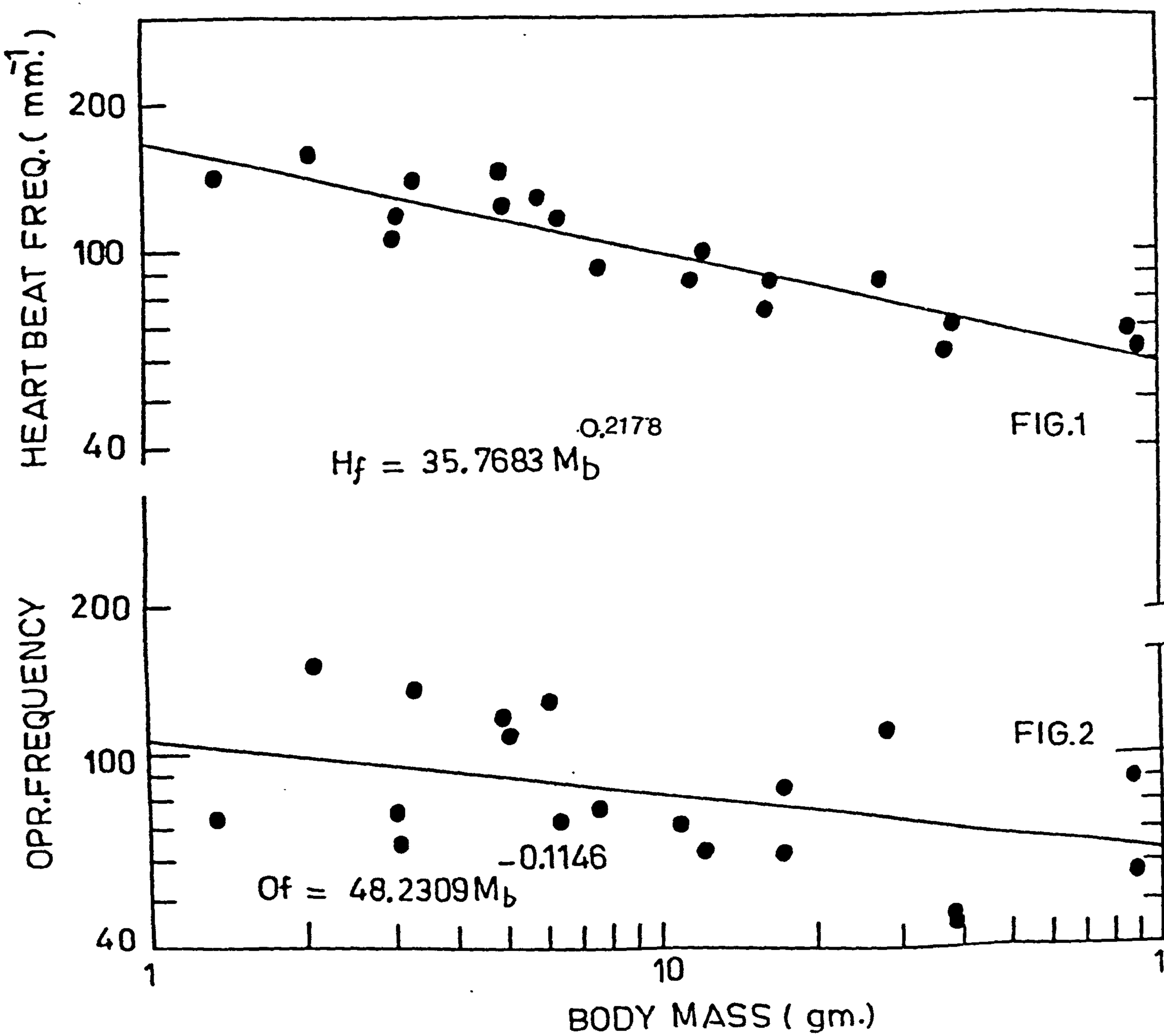
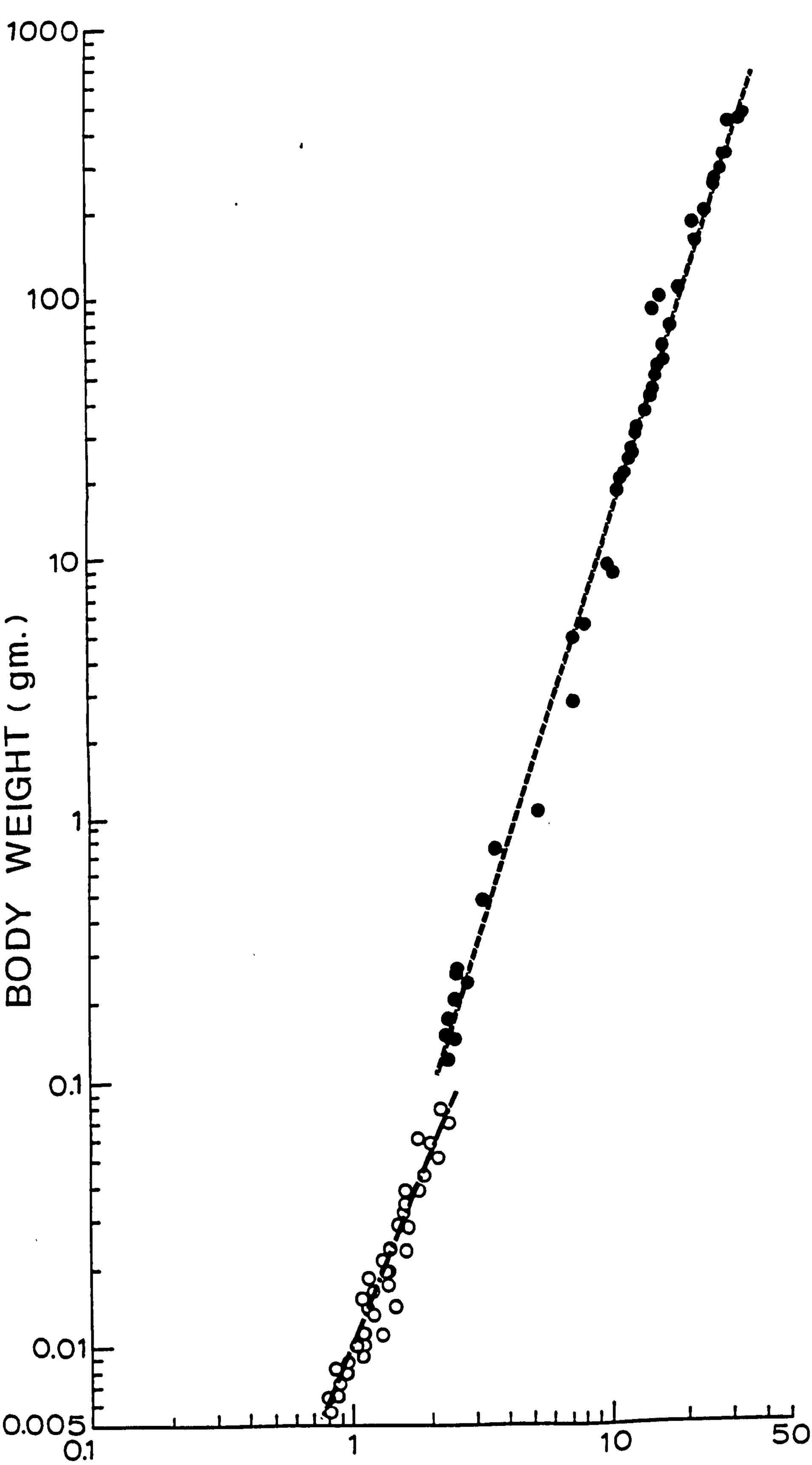

HEART BEAT FREQ.(mm.$^{-1}$)
200
100
40
$H_f = 35.7683\, M_b^{0.2178}$
OPR.FREQUENCY
200
100
40
$Of = 48.2309 M_b^{-0.1146}$
1
10
100 X 10^{-4}
BODY MASS (gm.)

FIG.1

FIG.2

FIGURE 21

Bilogarithmic plot between
body length v. body weight


1000
100
10
1
0.1
0.01
0.005
WEIGHT (gm.)
0.1
1
10
50
BODY LENGTH (cm.)

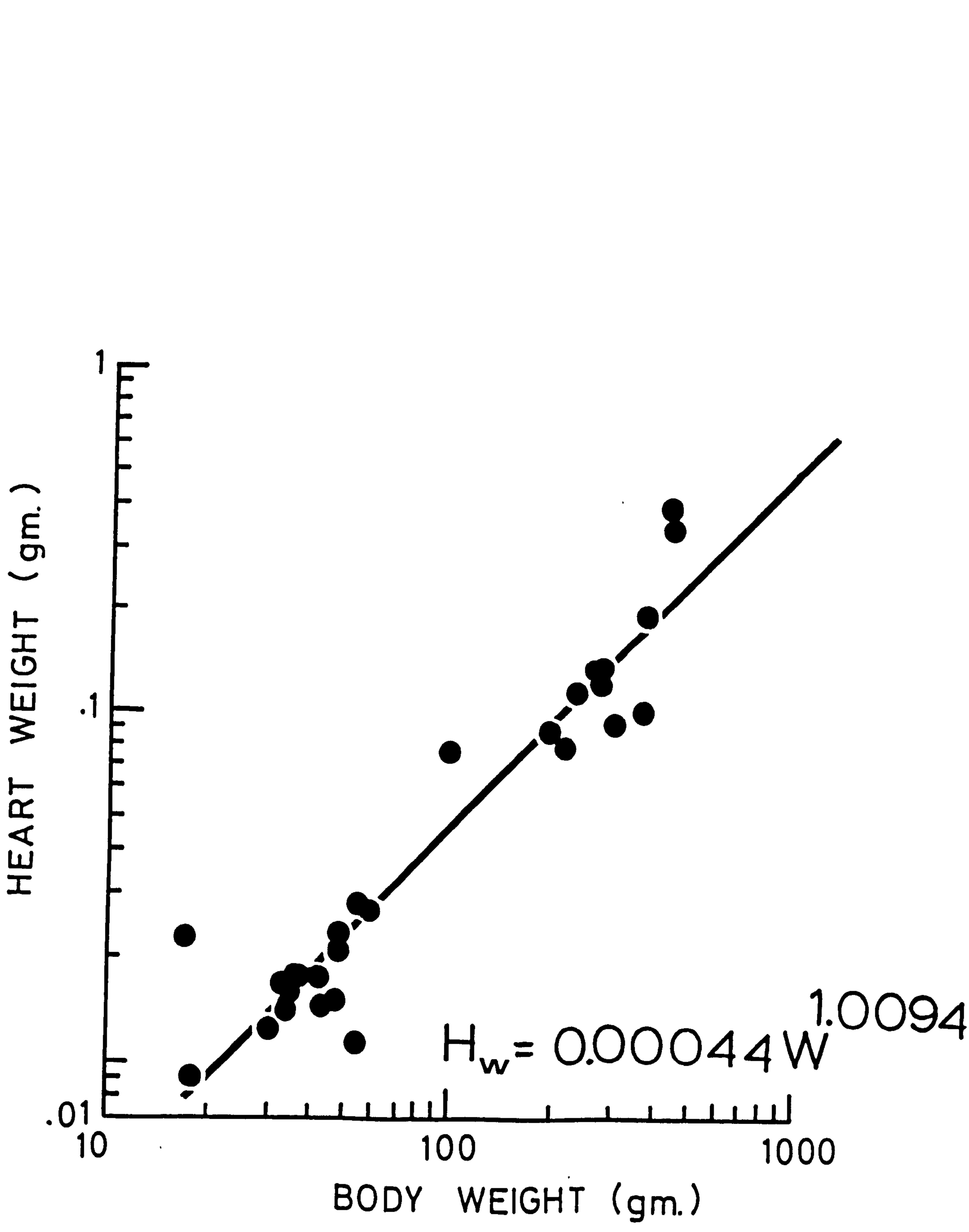
Stages	Equations	Corr. coef.
Young stages	$W = 0.0097\ L^{2.3755}$	0.9528
Adult stages	$W = 0.0119\ L^{2.9636}$	0.9969

6.3.6. Heart Weight in relation to Body Weight

When the data of heart weight of P. flesus were plotted on log-log coordinates, they gave a straight line with a slope of 1.0094 (Table 21, Fig. 22). The correlation coefficient between two parameters showed high correlations ($r = 0.9503$; $P < 0.001$).

FIGURE 22

Bilogarithmic plot between
heart weight v. body weight


HEART WEIGHT (gm.)
1
.1
.01
10
100
1000
BODY WEIGHT (gm.)
$H_w = 0.00044 W^{1.0094}$

6.4 DISCUSSION

6.4.1 Embryonic Development

Fish eggs present great diversity, not only in size and shape, but in the manner in which various life processes are coupled with their development. Variations are reported on the quantity of food present in the egg for its future development.

Platichthys flesus has a large quantity of yolk, which provides nourishment to the developing embryos in the early part of the life cycle. P. flesus receives its nourishment through the yolk-sac until the embryo reaches an age of 11 days. At this stage the mouth opens and the digestive system starts functioning. This time is very critical for the developing embryos due to the fact that they should be provided with appropriate and adequate food. Rotifers nauplii are suitable food for the earliest stages of P. flesus. To maintain the rotifer stocks, they are fed with algae. Therefore, a sort of food chain is established to feed fish with rotifers which consume cultured algae. The energy trapped by the algae is transformed to rotifers and from there to the embryos in the form of food.

Development, differentiation and functioning of respiratory organs are all important events in the

early developmental stages of fish. The development of respiratory organs should be synchronised with the development of cardio-vascular system and the digestive units and feeding of the embryos and larvae. Higher mortality which occurred during the life cycle of flounder was at the time when the embryos started feeding. This is because of the fact that the oxygen supply fails to provide adequate energy for the total metabolic activities of the fish.

In P. flesus the differentiation of gill arches starts at the age of six days, but they are not functional until they attain an age of 22 days. During this early part of life, the larvae get oxygen through cutaneous respiration. Development of scales seems to be an important factor, which initiates gill organisation and functioning as the diffusing capacity of skin decreases. Between 14-22 days seems to be the period associated with the organisation and development of scales on the skin. Indication of the functioning of the gills is found in the 22 day old larvae when the afferent and efferent filament arteries are joined together with transverse connections. The connections are the future marginal channels of the secondary lamellae. Other blood channels of the secondary lamellae develop from the flanges of pillar cells. Several studies (Camatini and Vailati, 1966; Camatini and Lanzavecchia, 1966)

ascribe an endothelial nature to the pillar cells. Early EM studies (Hughes and Grimstone, 1965) tended to doubt this view as secretion of collagen of the basement membrane and columns, and the possible contractility of pillar cells are not usual functions of the endothelium. Suggestions have also been put forward for the development of pillar cells from the smooth muscle cells of blood vessels (Munshi and Singh, 1968). The present study shows that the marginal channels of the secondary lamellae develop from the primordial vascular units and are, therefore, lined with endothelium. However, other blood channels are lined with a flange of pillar cells, which are mesenchymal in origin. This finding supports the views of Morgan (1973) that pillar cells differentiate directly from secondary mesenchyme cells. Present ultrastructural studies on the development of gills confirm this assumption.

Out of the various circulatory (arterio-arterial, arterio-venous and inter-filamentar) pathways of the gills, only the arterio-arterial pathway was clear in the sections of the larvae at the age of 55 days. The central venous sinus appears at later stages (88 day old larvae). After 88 days, the gill filaments are well developed and require oxygen and nourishment for their internal tissue. Internal

tissue of the gill filaments get oxygen and nourishment from finer capillaries originating from the efferent filament arteries (described in detail in Chapter 4). The central venous sinus develops from the primordial vascular units as it is lined internally by endothelium.

6.4.2 Heart beat frequency

The present study on the heart beat frequency of P. flesus indicates decreasing rate of the heart beat frequency during development. Decreasing heart beat frequency during development also has been recorded by Grodzinski (1948), Holeton (1970) and Morgan (1971). The differences in the heart beat recorded for various fish may result from the different conditions under which observations were made.

Temperature and intensity of light play an important role in the determination of the heart beat frequency/time. In the early stages of P. flesus the heart beat frequency decreases by a power of -0.2205. The heart beat frequency, however, becomes stabilised after the metamorphosis of young stages into adult one.

C H A P T E R 7

GILL VENTILATION IN THE FLOUNDER,

PLATICHTHYS FLESUS (L.)

Chapter 7 : GILL VENTILATION IN THE FLOUNDER, PLATICHTHYS FLESUS (L.)

7.1 INTRODUCTION

Ventilation is one of the important physiological factors which influences the capability of respiratory organs. Gill ventilation in teleosts is under the influence of buccal pressure and opercular suction pumps (Hughes & Shelton, 1957). The visceral skeleton, under the influence of co-ordinated contraction of a series of cranial muscles, operates the respiratory pumps.

The processes involved in gill ventilation have attracted the attention of many ichthyologists in the past (Baglioni, 1907; 1910; Willem, 1931; 1940; 1947; Woskoboinikoff, 1932; Van Dobben, 1937; Kirchhoff, 1958). These studies were based mainly upon anatomical details and therefore failed to show the exact mechanism of gill ventilation. With the advent of electrophysiological technique, however, the mechanics of gill ventilation have been demonstrated in many fish species (Hughes & Shelton, 1957; 1958; 1962; Ballintijn & Hughes, 1965; Hughes, 1973). Little, however, is known of the morphological adaptations connected with the gill ventilation of the flatfish (Yazdani & Alexander, 1967; Yazdani, 1976; Douglas & Lanzing, 1981).

The present study is an attempt to examine the

morphological and physiological adaptations concerned with gill ventilation in the Flounder, Platichthys flesus.

7.2 MATERIALS AND METHODS

Fish for this study ranged in size from 50-200g, which were collected from the stocking concrete tank of 5x1x1 metre capacity of the Marine Biological Association Laboratory and kept in the aquarium at the Zoology Department, Bristol University.

7.2.1 Skeletal Elements

The skeletal elements concerned with gill ventilation were removed from the head of the fish and immersed in warm water containing 1% NaOH. After thorough cleaning from the adhering muscles, the bones were washed in tap water to remove traces of NaOH and finally were transferred to a mixture of equal parts of H_2O_2 and water for final inspection.

7.2.2 Anatomy

The gills and opercular apparatus of pre-metamorphic stages were fixed in 5% glutaraldehyde in 0.2M cacodylate buffer at pH 7.4.

Post-metamorphic stages were fixed in aqeous seawater Bouins fluid.

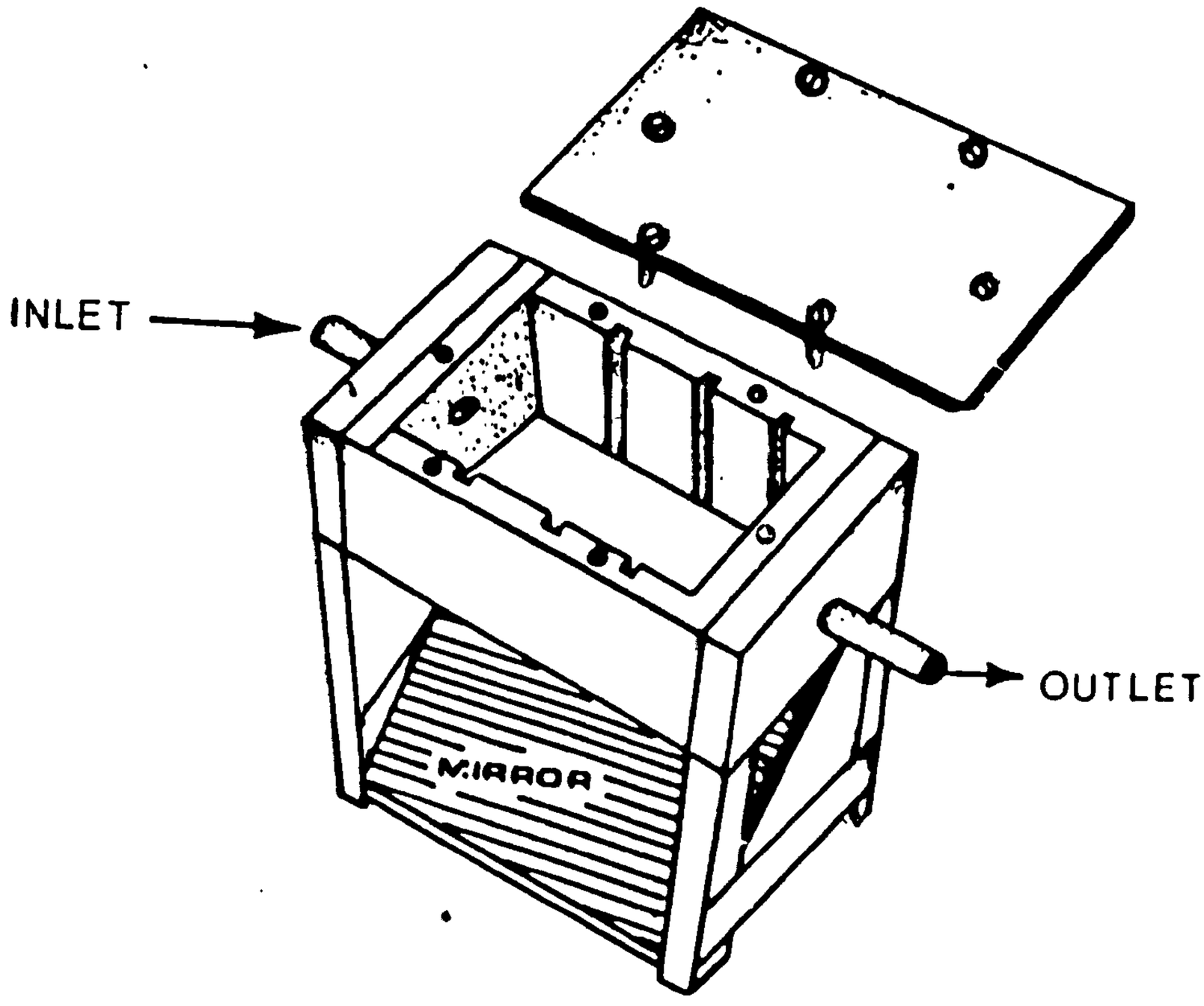
Small and large fish were embedded respectively in araldite and paraffin wax to obtain serial sections through the branchial regions. Semi-thin serial araldite sections (1 μm) of the pre-metamorphic stages and (5 μm) paraffin serial sections of the post-metamorphic stages were stained in 1% Toluidine blue and Harris haematoxylin for detailed microscopical examinations.

7.2.3 Experimental set-up

Ventilatory currents in pre- and post-metamorphic stages were studied by directing Indian Ink diluted in saline to the inspired water currents. For pre-metamorphic (larval) stages, the ventilatory currents were observed through a binocular microscope. In the post-metamorphic (adult) stages, the part played in gill ventilation by the lower opercular cavity was observed through a mirror fitted to the floor at an angle of 45^{o} to the experimental set-up as shown in Figure 23.

FIGURE 23

Diagram of the apparatus used for assessing ventilatory movements of upper and lower opercular cavities of P. flesus


INLET
MIRROR
OUTLET

7.3 RESULTS

Pre-metamorphic stages of P. flesus are free-swimming. At this stage, the gills and opercular cavities of the upper and lower sides are symmetrical and equally developed (Fig. 24). However, after metamorphosis, the fish becomes to a large extent bottom-dwelling. Normally it is the left side that rests on the substratum. This change in the behaviour of the fish may bring about the changes in the anatomical details of the entire respiratory apparatus. Such changes may include:-

1. The osteological details of the opercular bones of the two sides showed differences. The bony elements of the lower side being smaller and flatter than those of the upper side (Fig. 25).

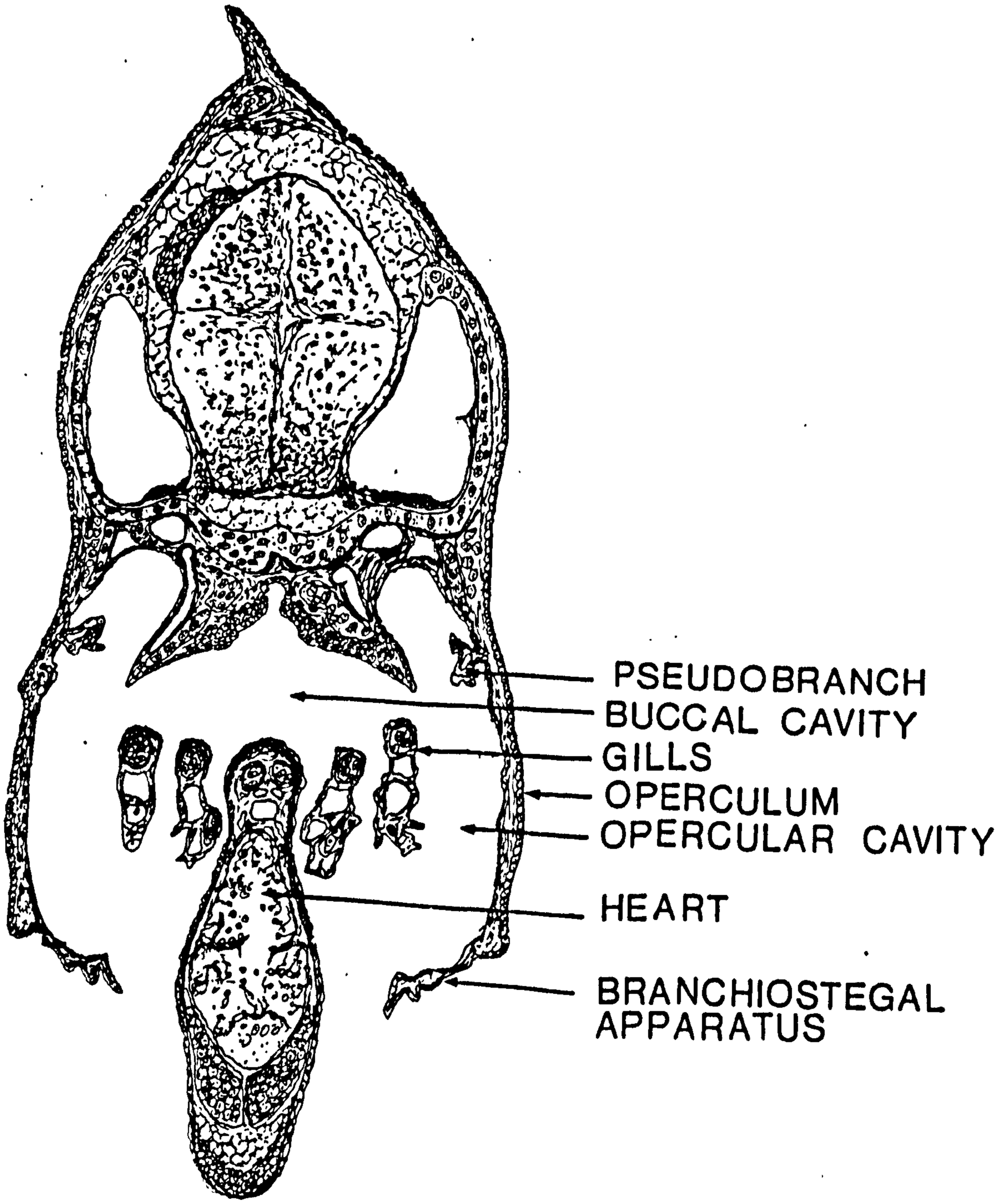
2. The surface area of the upper cavity is greater than the lower cavity at different distances from the tip of the head (Fig. 26).

3. Smaller dimensions of the gills in the lower opercular cavity in comparison with those of the upper cavity, associated with the flat nature of the lower side (Fig. 27).

In pre-metamorphic (symmetrical) stages, water currents are expelled through both the opercular openings.

FIGURE 24

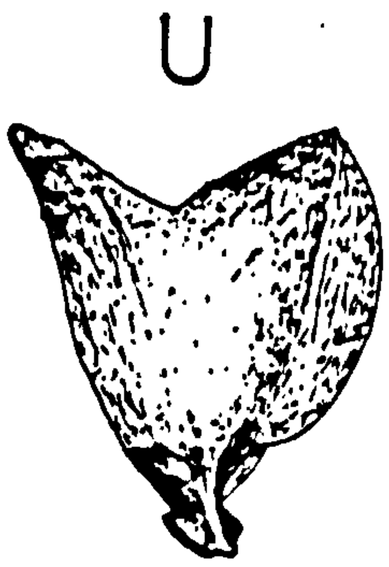
Drawing of a transverse section through head of a 23-day old flounder before metamorphosis. Note the symmetrical opercular cavities, opercular curvation and gills of both sides.

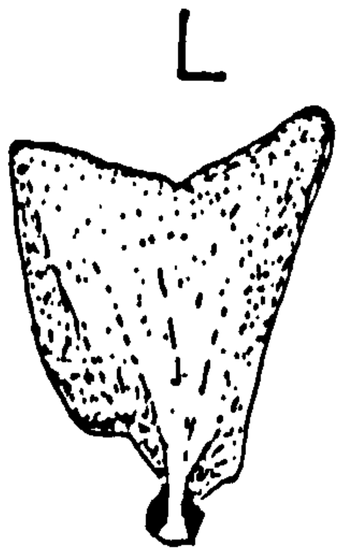

PSEUDOBRANCH
BUCCAL CAVITY
GILLS
OPERCULUM
OPERCULAR CAVITY
HEART
BRANCHIOSTEGAL APPARATUS

0.25 mm.

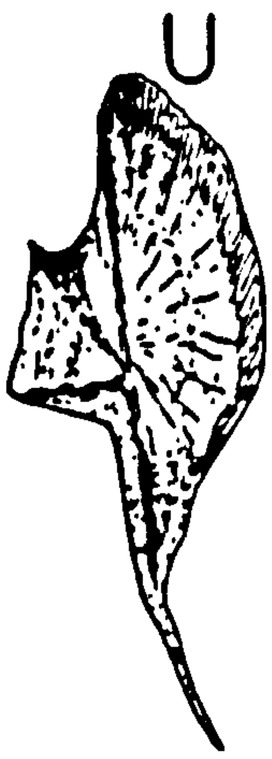
FIGURE 25

Drawing showing that the opercular bones of lower side (L) are smaller and flatter than those from the upper operculum (U). The V-shaped urohyal bone is also shown.

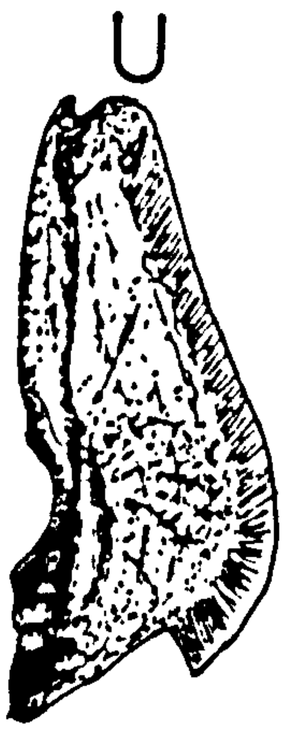


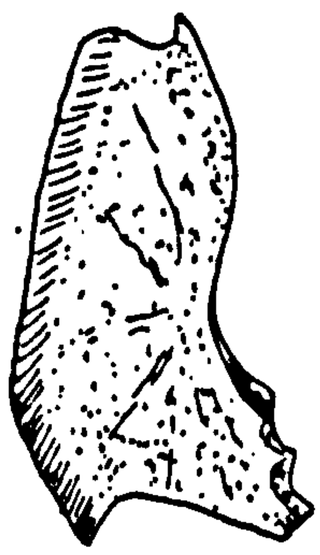


opercular

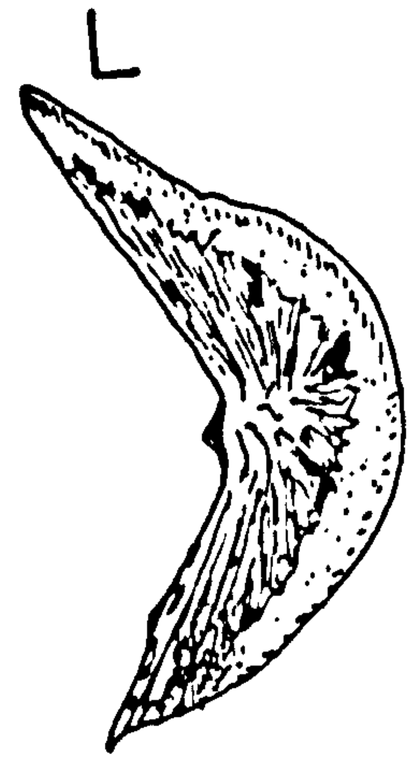


subopercular

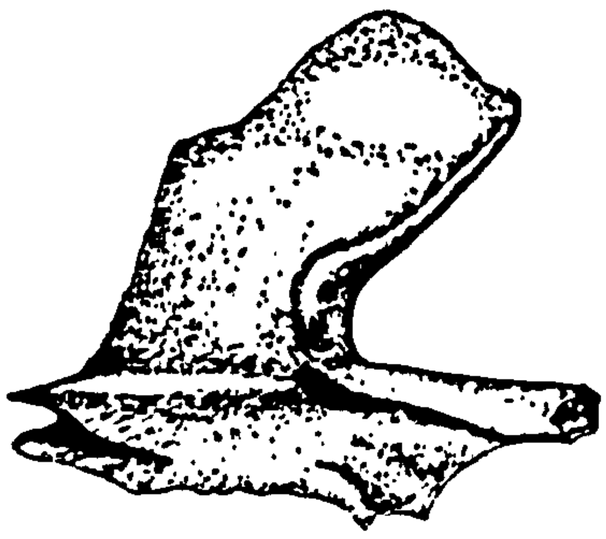




interopercular



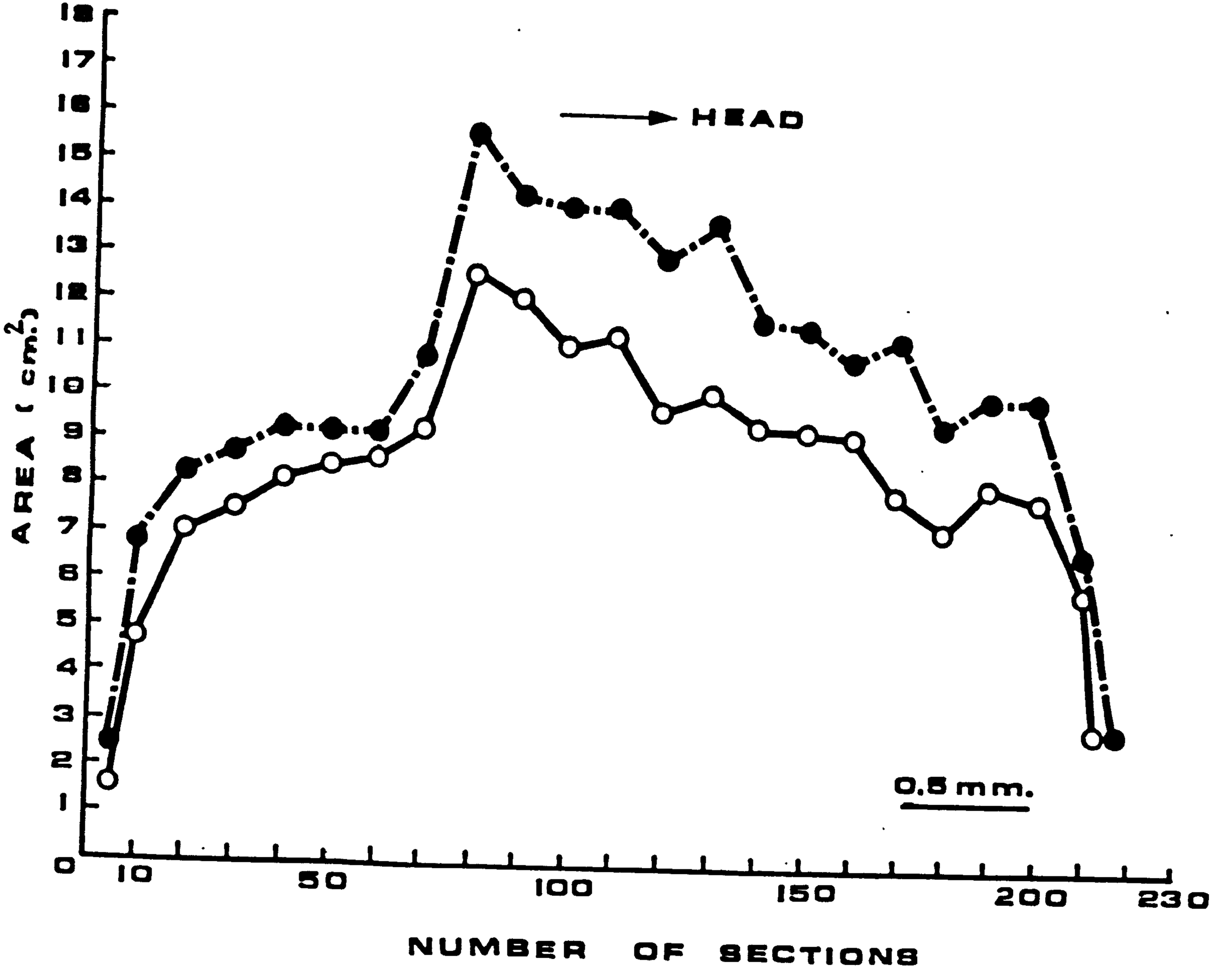
preopercular

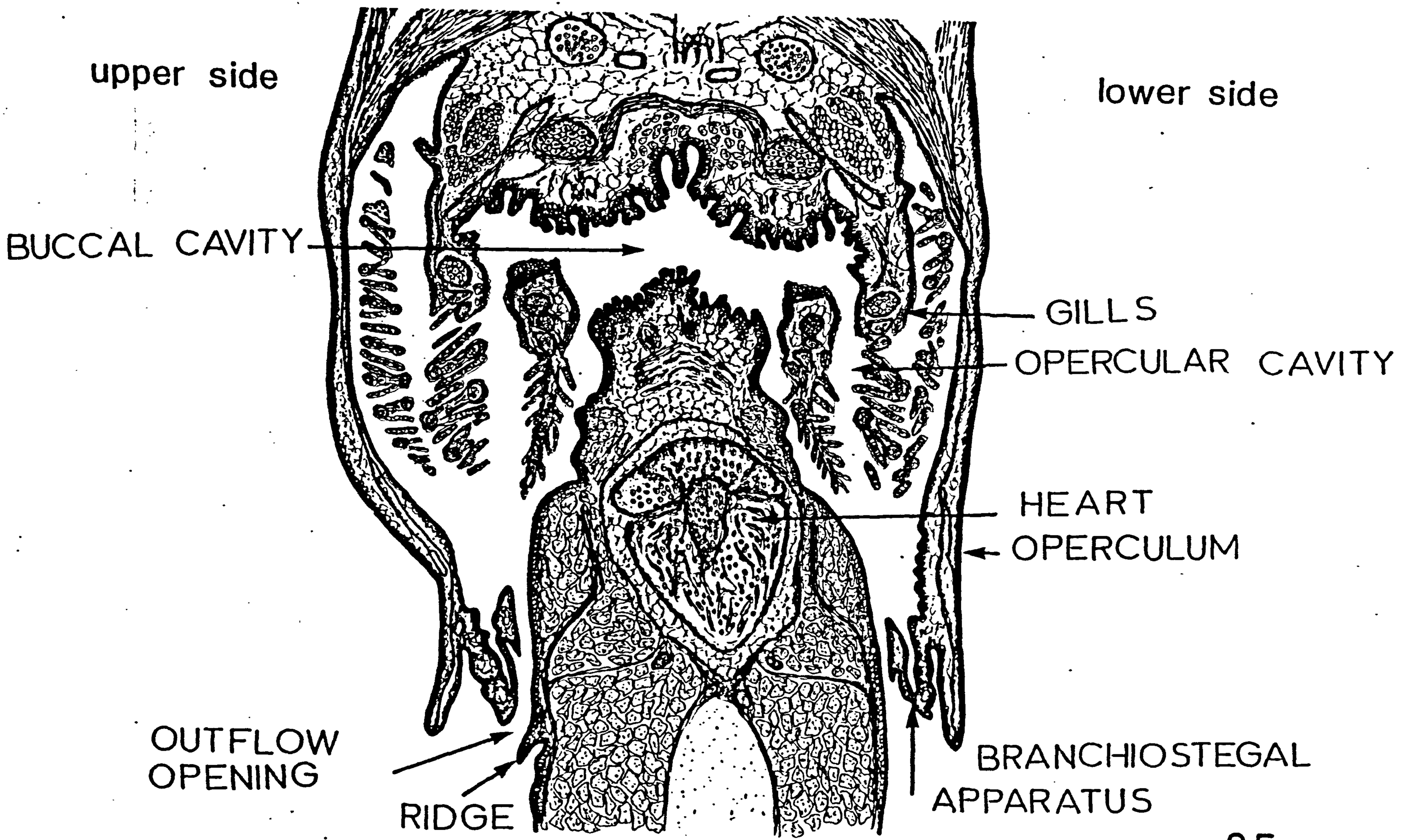


UROHYAL

FIGURE 26

Plots showing area of cross sections of upper (●--●), and lower (o—o) cavities at different distances from the tip of the head.


HEAD
AREA (cm.²)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0.5 mm.
10
50
100
150
200
230
NUMBER OF SECTIONS


upper side
lower side
BUCCAL CAVITY
GILLS
OPERCULAR CAVITY
HEART
OPERCULUM
OUTFLOW OPENING
RIDGE
BRANCHIOSTEGAL APPARATUS
0.5 mm.

The amplitude of the opercular movements on both sides are equal. At rest, the post-metamorphic (asymmetrical) stages expel ventilatory water through the upper opercular opening (Pl. 19a). A small volume of ventilatory water is expelled through the lower opercular opening when the fish moves or is excited. Variations in the amplitudes of the two opercular movements have been observed in adult stages.

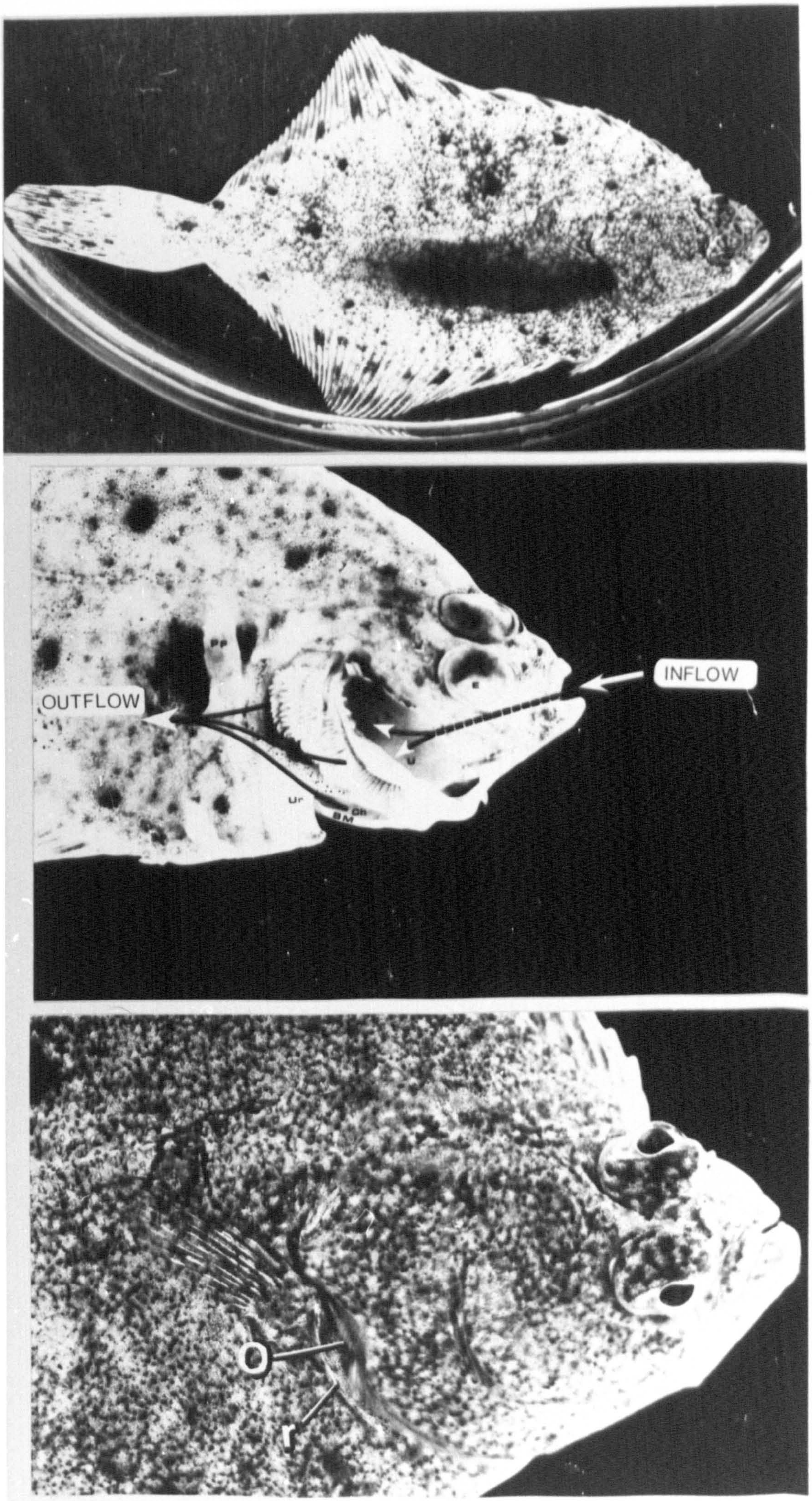
The ventilatory current, which enters through the mouth (inflow water) is directed towards the upper and lower opercular cavities. The water from the lower opercular cavity appears to be directed dorsally via a communicating channel and expelled outside (outflow water) (Pl. 19b) through a narrow opening surrounded dorsally by the branchiostegal membrane and ventrally by a ridge. This may play an important part in directing water current dorsally to avoid the disturbance of sediments, which usually cover the body except the eyes, mouth and dorsal opercular opening (Plate 19c).

PLATE 19

a. Photograph of a live specimen showing that dye mixed with the inspired current is expelled through a narrow opening on the upper side.

b. Photograph of the head region with the right operculum removed. Arrows indicate the direction of ventilatory water flow through the lower (L) and upper (U) opercular cavities, which communicate by a channel (Ch) enclosed by V-shaped urohyal bone (ur) and branchiostegal membrane (bm), pectoral fin (pf), eye (e).

c. Photograph of the head region (live specimen) showing the opening for expulsion of water (o) on the upper side, ridge (r) is also shown.


INFLOW
OUTFLOW
Ur
GR
BM
O
r

7.4 DISCUSSION

Anatomical studies of gill ventilation in P. flesus reveal variations in the course of ventilatory currents in pre- and post-metamorphic stages. Such differences in the ventilatory currents are likely to be related to variations in the body shape in the two stages.

The pre-metamorphic stages are free-living and their body configuration is similar to that of typical teleostean fish. In these forms, the opercular cavities, the gills and the bony elements concerned with gill ventilation are equally developed on both sides and, therefore, the buccal pressure and the opercular suction pumps force equal volumes of water with the same velocity through the two opercular cavities. However, the adult fish shows differences in the opercular cavities, gills and bony components concerned with gill ventilation, between the two sides. The more concave upper opercular cavity is larger and accommodates better developed gills than the flat and smaller lower opercular cavity with moderately developed gills. Changes are due to the metamorphosis of active and free-swimming young stages to the bottom-dwelling and sluggish adult forms.

When the fish are at rest, the buccal pressure pump forces water into the two opercular cavities. Because of the greater depths of the dorsal opercular cavity,

the water current from the ventral side is directed towards the dorsal side and finally expelled out through the upper opercular opening. When the fish is excited, however, the suction created by the lower cavity matches that of the upper cavity and, therefore, an equal volume of water is expelled through the lower and the upper opercular cavities.

The expulsion of water mainly through the upper opercular opening is well suited to the bottom-dwelling habit of the fish. The lower opercular opening faces the bottom of the water column and therefore one could postulate that a considerable force is required to force water through the sandy or muddy bottoms of the estuaries.

Present findings on the ventilatory currents in adult specimens corroborate the earlier findings in flatfish (Yazdani and Alexander, 1967; Yazdani, 1976). However, these studies do not mention the variation in the ventilation current in the pre- and post-metamorphic stages of flounders. The electrophysiological experiments on the movement and pressure of both opercula of Pleuronectes platessa showed identical recording (Hughes, 1960). Resistance of water flow is greater in gills where the epibranchial meets the ceratobranchial. Greater resistance is due to higher frequency of filaments at this region. The number of

filaments also differs in the two respective hemibranchs of the upper and lower hemibranchs.

C H A P T E R 8

GENERAL DISCUSSION

CHAPTER 8 GENERAL DISCUSSION

8.1 INTRODUCTION

Although flounders have many structural features specialised in relation to their environment, nevertheless the basic morphology of the gas exchange surfaces as shown by electron microscopic studies (Chapter, 4) is similar to that found in other fish. The development of these structures and other parts of the gill system is also similar to that in rainbow trout, Salmo gairdneri (Morgan, 1971) which appears to be the only other fish which has been studied from this point of view. Morphometric studies of the development of different parts of the gill system have also extended these similarities although the slope of the regression lines for gill area/body weight of the flounder (0.824) is less than that of the rainbow trout (0.95)but is very close to that of the small-mouthed bass, Micropterus dolomieu studied by Price (1931). As can be seen from Fig. 28, the regression lines for flounder, bass, and rainbow trout are very close to one another. Flounder has a larger gill area than that of either Torpedo or Anabas for fish of the same weight.

The relationship between gill area and body weight of the flounder is also similar to that of other flatfish that have been investigated (Fig. 29). The

FIGURE 28

Bi-logarithmic plots showing relationships between body weight (gm.) v. total gill area (cm^2) for a number of fish species.

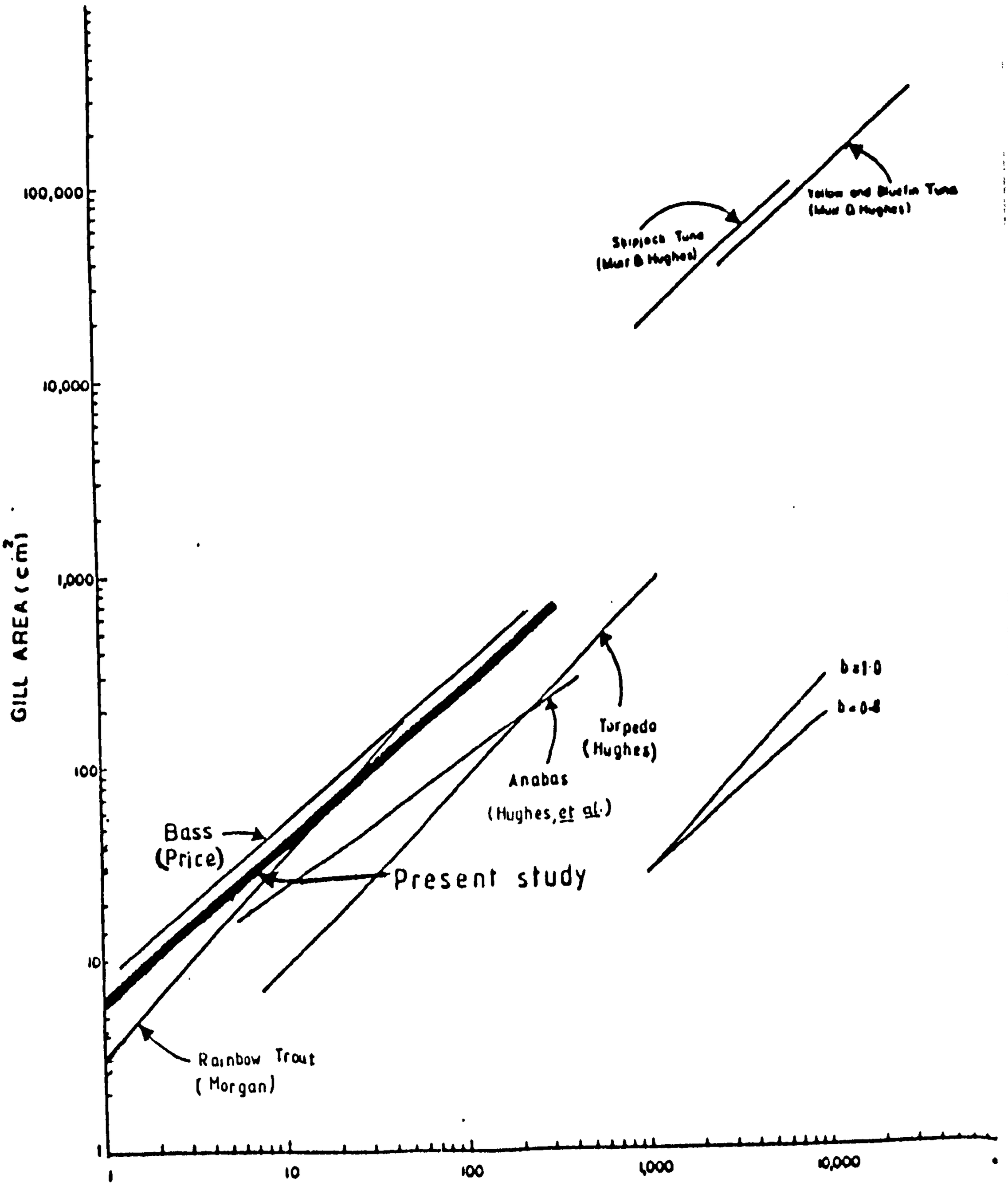
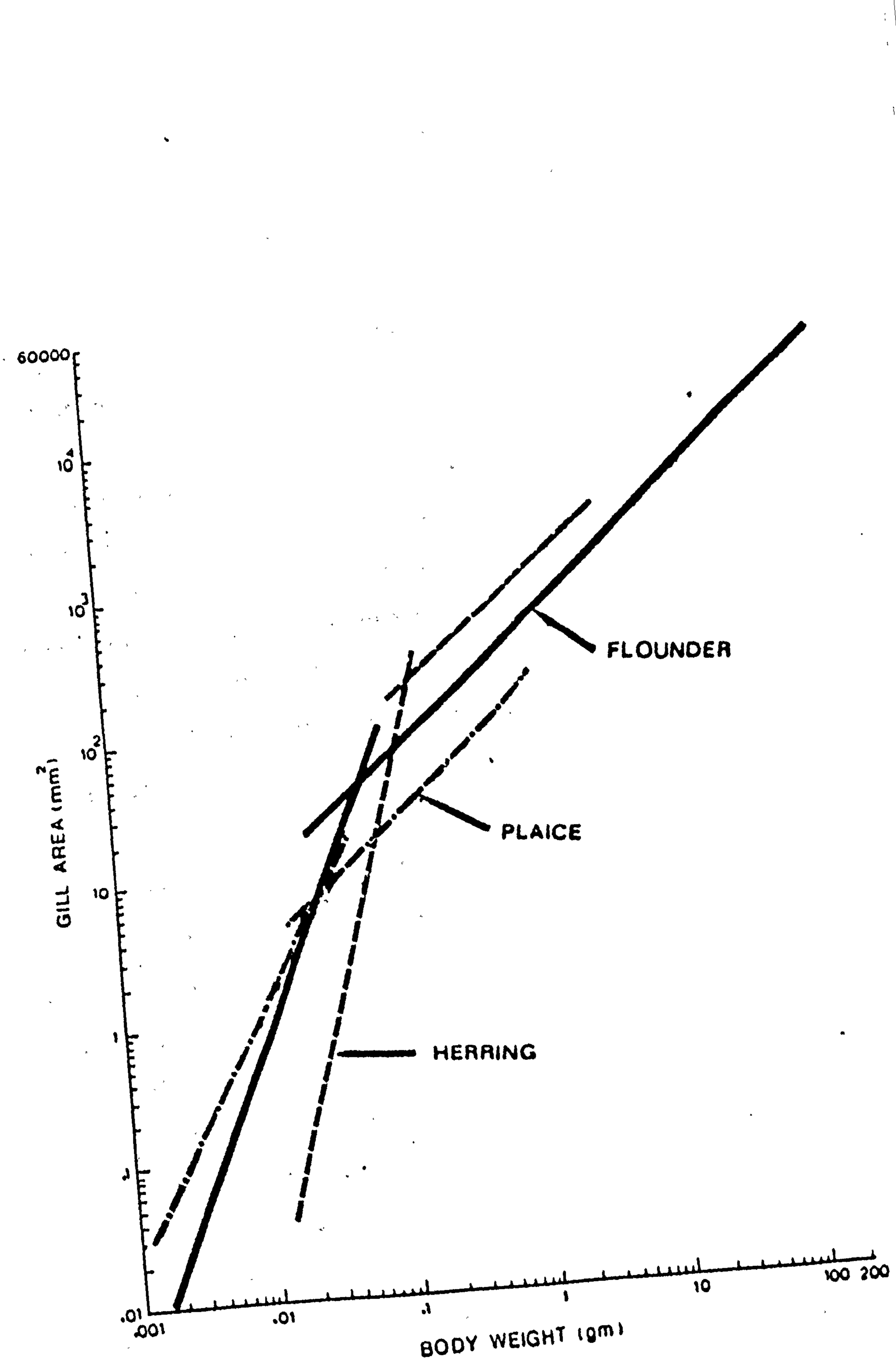

Skipjack Tuna
(Hunt & Hughes)
Yellow and Bluefin Tuna
(Hunt & Hughes)
Torpedo
(Hughes)
Anabas
(Hughes, et al.)
Bass
(Price)
Present study
Rainbow Trout
(Morgan)
b = 1·0
b = 0·8
GILL AREA (CM²)
100,000
10,000
1,000
100
10
1
1
10
100
1,000
10,000
BODY WEIGHT (gm.)

FIGURE 29

Bi-logarithmic plots showing relationship between body weight (gm.) v. gill area (mm^2) for herring, plaice,(De Silva, 1974) and flounder. Note the inflexion of regression lines during the development.


60000
10^4
10^3
10^2
10
1
.01
.001
AREA (mm²)
GILL
FLOUNDER
PLAICE
HERRING
.01
.1
1
10
100 200
BODY WEIGHT (gm)

slope for flounder (0.824) being close to that for the plaice, Pleuronectes platessa (0.85). At very early stages in development the similarity is even greater because both species have an inflexion in the log/log relationship between gill surface area and body weight. As has been discussed in Chapter 5 this inflexion is associated with a marked metamorphosis of the whole respiratory system: before metamorphosis the slope is much greater than during post-metamorphic development of the gill system. Gill area increases very rapidly during the pre-metamorphic stages, the slopes for plaice and flounder being respectively 1.59 and 2.213. These inflexions occur for fish of about 0.02- 0.05g body weight.

The association of an inflexion with metamorphosis from a roundfish type of organisation to that characteristic of flatfish is perhaps not surprising. However, the existence of such an inflexion in the area/weight regression has also been found in the herring at a body weight of about 0.1g (De Silva, 1974). Although very few fish species have been studied from this point of view the herring would appear to be the only roundfish which has a marked inflexion. The slope of the regression line in the pre-inflexion phase (b = 3.36) is also much steeper than that for the two flatfish species. Close inspection of data plotted for rainbow trout (Morgan, 1971) shows gill areas of

specimens below 0.1g which were omitted from the regression calculation. These points suggest, however, the possibility of an inflexion, but more detailed study would be necessary to establish this for trout.

It is of interest to consider the significance of these inflexions in the growth of flounder and several features are relevant in this context. In relation to gas exchange the early stages of development are characterised by a high surface/volume ratio so that much gas exchange can take place through the body surface. With increase in size the surface becomes less capable of supplying sufficient oxygen and this is associated with development of the gills. Thus, in the earlier stages of development a much more rapid rate of increase in the gill surface area would compensate for the reduction in cutaneous surface. Although the herring is the only roundfish in which a clear inflexion has been demonstrated for the development of the gill system, changes in rate of development of respiratory surfaces in relation to body size have been encountered both in roundfish and in airbreathing species. For example, in the sea bream studied by Iwai & Hughes (1977) there was a significant change in the surface/volume relationship of the gills themselves although this was at a much later stage in development than that shown for the flounder. Inflexions in bilogarithmic plots have also been described(Hughes, et al, 1974)

for airbreathing species in relation to the development of the airbreathing organ and these are related to changes in the dependence of the fish on aquatic and aerial respiration.

The interpretation of the inflexion in the flatfish curves might well be related to the respiratory requirements of the fish during development and perhaps measurements of the surface area of the fish would reveal interesting changes which might be correlated with the gill area development. It must also be remembered that in terms of gas exchange, changes in thickness of the water/blood barrier are also of importance both with respect to cutaneous and gill oxygen uptake. Furthermore, development of the blood system is a vital part in this whole process and the appearance of haemoglobin to help in transport of oxygen away from the respiratory surface is another important aspect. Red blood cells containing haemoglobin seem to appear in flounder at a body size of about 0.0035g i.e. well before metamorphosis.

Other interpretations of the inflexion might be related to changes in nutritional and metabolic processes and these have been invoked in relation to later stages in the development of yolky eggs, particularly the change from a dependence on the yolk to the metabolism of food ingested by the developing fish.

There is of course the possibility that the inflexion is more related to specific changes which occur at metamorphosis in flatfish. These changes are certainly significant for not only are there changes in external form of the fish and some anatomical features of the respiratory system (Chapter, 6), but also there is the transition from a pelagic to a bottom-living habitat. With respect to respiratory requirements the pelagic stages live in water which is more or less saturated with oxygen and O_2 uptake could take place over the whole body surface as well as the developing gills. When the fish lives on the sea bottom, however, cutaneous oxygen uptake is probably reduced because of a thickening of the skin,including development of scales, and there is the possibility of the inspired water being low in oxygen. Many of the adaptations of the gills and ventilatory mechanisms (Chapter, 7), seem to enable flounder to obtain water from above the mud surface and prevent the entry of mud into the respiratory cavities which would not only reduce the oxygen content of water passing the gills but might damage the gills physically.

The overall nature of the results described in this thesis show that there are many adaptations of the respiratory system of flounder some of which have previously been emphasised with respect to the adult fish. It is now apparent that many of these are even

more evident when their development is followed. The contrast between early developmental stages with their mainly free-living habit to adult fish mainly living on the sea bottom is a very great one and it is not surprising that such marked changes occur in the development of the respiratory system and which fully justify description as a "metamorphosis".

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CHAPTER 9

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Chapter 9 : REFERENCES

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A P P E N D I X 1

Tables

TABLE 1 : Summary of the table showing mean values of standard oxygen consumption of different weight groups of Platichthys flesus at 5°C

Body Weight (g)	O_2 Consumption ($\mu l\ O_2 \cdot h^{-1}$)	t = 2.11 * N = 19 **
0.0090	2.5830	
0.0092	2.0100	
0.0095	3.4320	
0.0100	2.2800	
0.0120	2.0110	
0.0135	3.4190	
0.0150	3.8000	
0.0150	2.5790	
0.0150	3.1880	
0.0165	4.6850	
0.0170	4.7790	
0.0220	7.2480	
0.0230	5.5100	
0.0280	6.3800	
0.0280	8.5770	
0.0330	8.8900	
0.0330	10.9800	
0.0350	6.8000	
0.0450	11.5000	

* Student's t variable

** Number of specimens

TABLE 2 : Summary of the table showing mean values of standard oxygen consumption of different weight groups of Platichthys flesus at 10°C

Body Weight (g)	O_2 Consumption ($\mu l\ O_2 \cdot h^{-1}$)	t = 2.069* N = 25 **
0.0038	4.2000	
0.0060	3.7720	
0.0075	4.1010	
0.0086	6.8000	
0.0100	5.1670	
0.0110	3.6178	
0.0120	9.0100	
0.0130	10.5710	
0.0130	10.7240	
0.0140	6.7113	
0.0150	6.4300	
0.0150	11.3350	
0.0150	6.9500	
0.0180	5.8810	
0.0190	11.8950	
0.0220	10.4000	
0.0220	18.4210	
0.0230	13.6219	
0.0310	14.0400	
0.0310	27.1200	
0.0360	13.5440	
0.0400	31.0400	
0.0490	28.8300	
0.0600	34.0530	
0.0609	29.5000	

* Student's t variable

** Number of specimens

TABLE 3 : Summary of the table showing mean values of standard oxygen consumption of different weight groups of Platichthys flesus at 15°C

Body Weight (g)	O_2 consumption ($\mu l\ O_2 . h^{-1}$)	t = 2.093* N = 21 **
0.0040	5.8030	
0.0044	6.5700	
0.0068	6.6000	
0.0068	5.8000	
0.0084	7.4133	
0.0090	8.1644	
0.0100	9.4460	
0.0100	10.0500	
0.0125	13.9910	
0.0130	12.6500	
0.0155	18.7290	
0.0155	14.0230	
0.0170	18.4000	
0.0170	22.8300	
0.0175	15.3000	
0.0180	10.5580	
0.0230	20.8300	
0.0260	22.0140	
0.0280	29.5100	
0.0370	32.7880	
0.0510	40.9300	

* Student's t variable

** Number of specimens

TABLE 4 : Summary of the table showing mean values of standard oxygen consumption of different weight groups of Platichthys flesus at 20°C

Body Weight (g)	O_2 Consumption ($ul\ O_2 . h^{-1}$)	t = 2.131* N = 17 **
0.0095	11.6550	
0.0110	14.5000	
0.0110	16.8700	
0.0140	15.7900	
0.0140	17.1770	
0.0150	18.5000	
0.0150	16.6300	
0.0190	21.7100	
0.0190	22.1700	
0.0200	26.4100	
0.0300	27.4200	
0.0300	31.4300	
0.0340	41.3500	
0.0440	45.0600	
0.0510	45.2200	
0.0560	49.0560	
0.0610	60.1240	

* Student's t variable

** Number of specimens

TABLE 10: Summary of table showing values for gill area and its component parameters in different weight groups of larval stages of Platichthys flesus L.

Body Weight (gm)	Total filament length(mm)	Frequency of secondary lam./mm (both sides of filament)	Total number of secondary lamellae/ fish	Bilateral area of an average secondary lam.(mm^2)	Total gill area (mm^2)	Total gill area mm^2/g Body weight
0.0021	2.06	55.800	114.9480	0.00015	0.0172	8.2106
0.0025	3.90	55.200	215.2800	0.00017	0.0366	14.0760
0.0030	5.10	52.400	267.2400	0.00021	0.0561	18.7068
0.0037	7.20	55.140	398.8800	0.00020	0.0794	21.4599
0.0040	5.00	53.000	265.0000	0.00020	0.0530	13.2500
0.0045	9.20	53.200	489.4400	0.00018	0.0881	19.5776
0.0050	14.90	51.800	771.8200	0.00012	0.0926	18.5237
0.0051	18.70	51.500	963.0500	0.00032	0.3082	60.4267
0.0065	16.20	50.600	819.7200	0.00038	0.3115	47.9921
0.0075	17.30	51.994	899.4962	0.00053	0.4767	63.5644
0.0090	30.20	49.600	1497.9200	0.00081	1.1986	133.1820
0.0100	31.60	50.400	1592.6400	0.00043	0.6848	68.4835
0.0120	60.40	48.400	2923.3600	0.00078	2.2022	190.0180
0.0190	39.50	46.800	1848.6000	0.00057	1.0537	55.4580
0.0320	47.20	48.200	2275.0400	0.03950	8.4864	280.8253
0.0320	66.03	49.000	3235.4700	0.00571	16.7874	399.7000
Mean 0.0105	23.41	51.440		0.00092	2.0270	88.3365

TABLE 11 : Summary of table showing values for gill area and its component parameters in different weight groups of adult stages of Platichthys flesus L.

Body weight (gm)	Total filament length(mm)	Frequency of secondary lamellae/mm (both sides of filament)	Total number of secondary lamellae/ fish	Bilateral area of an average secondary lamellae(mm^2)	Total gill area (mm^2)	Total gill area (mm^2/g) Body Weight
0.0746	123.6200	49.4120	6108.3114	0.01331	81.3020	1089.834
0.1554	189.4700	49.8300	9441.2901	0.02002	189.0150	1216.310
0.2220	199.3400	48.0000	9568.3200	0.02190	209.5460	943.902
0.4390	256.2000	48.9000	12528.1800	0.02250	281.8840	642.105
0.9650	222.3460	50.0653	11131.8192	0.02452	272.9520	282.852
1.0100	458.4500	50.0321	22937.2162	0.03612	828.4920	820.289
2.3000	585.4000	45.6000	26694.2400	0.04190	1118.4890	486.299
4.0000	636.0000	40.8000	25948.8000	0.06380	1656.6550	414.163
5.1000	1007.6560	43.1250	43455.1650	0.08140	3537.2500	693.579
8.8000	1641.8700	39.0000	64032.9300	0.05540	3547.7240	403.116
19.0000	2117.5200	39.6507	83961.1503	0.08438	7084.6510	372.870
19.4300	1928.0000	41.8600	80706.0800	0.08502	6861.6309	353.146
22.5400	2134.9730	35.8041	76440.7868	0.11525	8809.7860	390.851
40.0000	2687.3000	35.8000	96205.3400	0.14320	13776.6050	344.415
61.0000	3387.7300	32.2535	109266.1946	0.24919	27228.0318	446.360
.0000	4463.8000	31.2600	139538.3880	0.27594	38504.2230	222.568
Mean 22.3773	1377.4797	46.3269	51122.7604	0.08340	7124.2647	281.8574

TABLE 18 : Heart-Beat and opercular frequencies in different weight groups of juvenile *Platichthys flesus*.

Sl. No.	Body Weight	Heart-beat frequency (min^{-1})	Opercular frequency (min^{-1})
1	0.0014	141	72
2	0.0021	156	150
3	0.0031	115	66
4	0.0031	105	75
5	0.0034	140	132
6	0.0050	142	109
7	0.0051	124	118
8	0.0059	128	126
9	0.0064	116	71
10	0.0076	90	76
11	0.0117	86	71
12	0.0122	102	61
13	0.0166	75	85
14	0.0169	85	63
15	0.0283	86	110
16	0.0380	62	50
17	0.0394	70	48
18	0.0880	69	89
29	0.0906	63	65

TABLE 19 : Length (cm) and weight (gm) relationship in very young fish of P. flesus

Length (cm)	Body Weight (gm)
0.82	0.0053
0.76	0.0064
0.92	0.0077
0.85	0.0082
0.95	0.0088
1.00	0.0090
1.02	0.0100
1.10	0.0100
1.10	0.0110
1.30	0.0110
1.20	0.0130
1.12	0.0140
1.47	0.0140
1.09	0.0150
1.20	0.0160
1.37	0.0170
1.15	0.0180
1.35	0.0190
1.40	0.0190
1.30	0.0210
1.40	0.0230
1.60	0.0220
1.65	0.0230
1.50	0.0280
1.65	0.0280
1.57	0.0310
1.58	0.0340
1.60	0.0380
1.80	0.0380
1.78	0.0420
1.86	0.0430
2.00	0.0560
1.80	0.0600
2.28	0.0680
2.20	0.0760

TABLE 20 : Length (cm) and weight (gm) relationship in adult fish of *P. flesus*

Length (cm)	Body Weight (gm)
2.38	0.118
2.50	0.140
2.38	0.147
2.28	0.170
2.48	0.200
2.80	0.225
2.45	0.240
2.48	0.245
3.20	0.470
3.60	0.720
5.43	1.000
7.40	2.600
7.45	4.600
8.30	5.200
10.90	8.450
10.25	8.800
11.40	17.000
11.60	18.000
11.90	19.000
12.00	20.000
12.60	23.000
13.20	24.000
12.90	25.000
13.70	26.000
13.60	28.000
13.90	30.000
15.20	35.000
15.10	40.000
15.60	43.000
16.10	47.000
16.20	48.000
16.80	52.000
16.90	54.000
17.40	54.000

cont..

Täble 20 (continued)

Length (cm)	Body Weight (gm)
17.70	62.000
19.50	75.300
20.10	100.400
22.10	155.000
24.20	184.000
29.00	249.000
29.20	283.000
29.40	265.000
29.70	295.000
30.00	286.000
30.20	334.000
30.50	311.000
31.70	330.000
32.10	423.000
37.90	445.000
38.70	475.000

TABLE 21 : Summary of the table showing values for heart weight (gm) and body weight(gm).

Body Weight (gm)	Heart Weight (gm)
33	0.0165
17	0.0224
434	0.343
366	0.192
272	0.119
30	0.0123
427	0.3936
266	0.133
213	0.079
362	0.100
98	0.0761
274	0.1345
54	0.0115
36	0.0175
230	0.1135
18	0.009
19	0.0079
34	0.014
58	0.027
35	0.0155
42	0.0173
47	0.0149
299	0.092
48	0.023
37	0.0175
194	0.0875
43	0.0145
48	0.0205
54	0.0282
47	0.0199

A P P E N D I X 2

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Vascular pathways in the gill filaments of the flounder, *Platichthys flesus* L.

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This study is concerned with functional organization of some of the blood pathways in the gill filament of the flounder, *Platichthys flesus* L. The existence of two independent vascular pathways has been confirmed. The blood from the efferent filament artery (EFA) enters the central venous sinus (CVS) through very small blood vessels which are characterized by the presence of sphincter-like structures. The existence of an independent chamber of the CVS mainly full of white blood cells provides evidence of an independent lymphatic system connected to the CVS. Gill rays support the afferent side of a gill filament whereas plasma and an extensive network of nutritive blood vessels in the CVS supports the efferent part.

I. INTRODUCTION

Fish gills provide an interesting architectural plan of the respiratory and haemodynamic systems. The respiratory (arterio-arterial) and nutritive (arterio-venous) streams are the pathways by which blood circulates through the various components of teleostean gills. The respiratory path of the blood flow through the gills has been established (Allis, 1912; Goodrich, 1930; Mott, 1950; 1951; Müller, 1839; Munshi & Singh, 1968; Muir, 1970.). However, less is known of the non-respiratory (arterio-venous) pathway of blood in fish gills (Riess, 1881; Steen & Kruysse, 1964; Richards & Fromm, 1969; Vogel *et al.*, 1973; 1974; 1976; Laürent & Dunel, 1976).

The present study is an attempt to demonstrate the functional organization of the various pathways in the gill filaments of a bottom-living marine flatfish.

II. MATERIALS AND METHODS

Specimens (5–200 g) of *Platichthys flesus* were obtained from the Plymouth Laboratory of the Marine Biological Association. The fish were anaesthetized in MS222 (0·01 g l^{-1}) and gill filaments were fixed at 0–4° C for 1 h in 5% glutaraldehyde with collidine, washed and post-fixed for 1 h in 1% osmium tetroxide both were buffered at pH 7·4 with marine teleost saline (Young, 1933).

Fixed materials were embedded in araldite and 1 μm thin transverse and sagittal serial sections of gill filament were obtained using an LKB Ultrotome III and stained in 1% Toluidine blue and 1% azure in 1% borax (Richardson, Jarret & Finke, 1960).

III. RESULTS

THE ARTERIO-ARTERIAL PATHWAY

The arterio-arterial pathway in the gills is mainly involved in conducting the venous blood to the secondary lamellae for gaseous exchange. It includes the afferent branchial artery, blood channels in the secondary lamellae, efferent filament artery and the efferent branchial artery.

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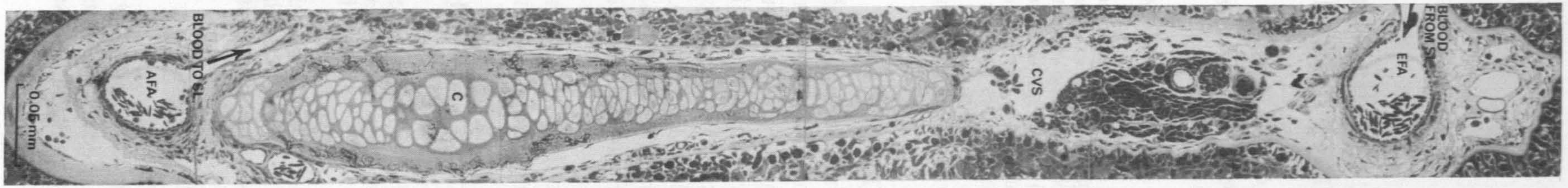

FIG. 1. Transverse section of a gill filament showing the afferent filament artery (AFA), efferent filament artery (EFA), cartilaginous gill ray (C) and the central venous sinus (CVS). Arrows indicate the direction of the blood flow to and from the secondary lamellae (SL). Epithelial layer (EP).

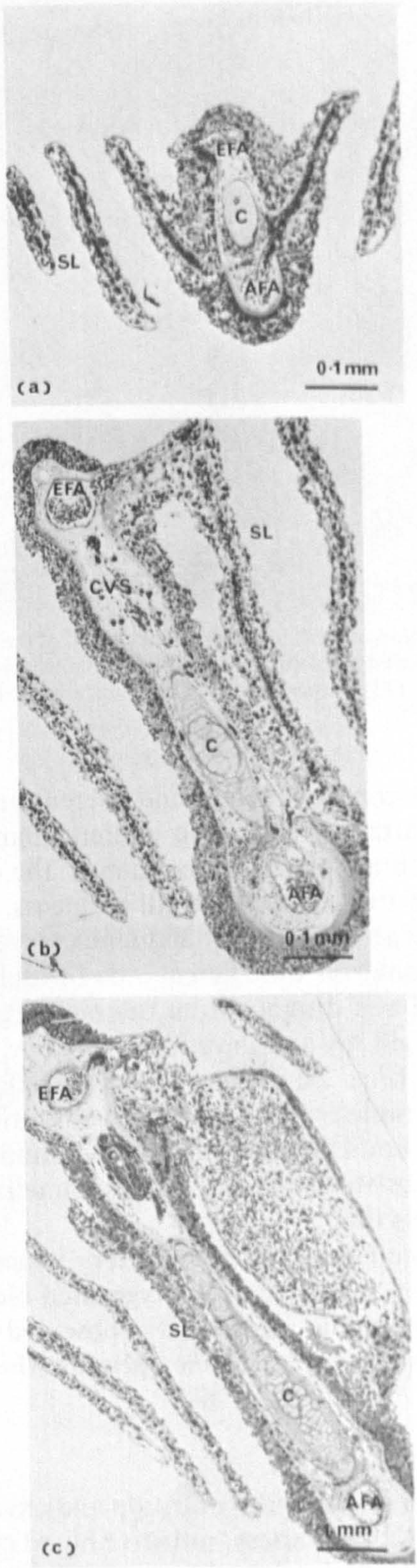

FIG. 2. Transverse sections of a gill filament from (a) tip; (b) middle and (c) base. Afferent filament artery (AFA); efferent filament artery (EFA); cartilaginous gill ray (C); secondary lamellae (SL); central venous sinus (CVS).

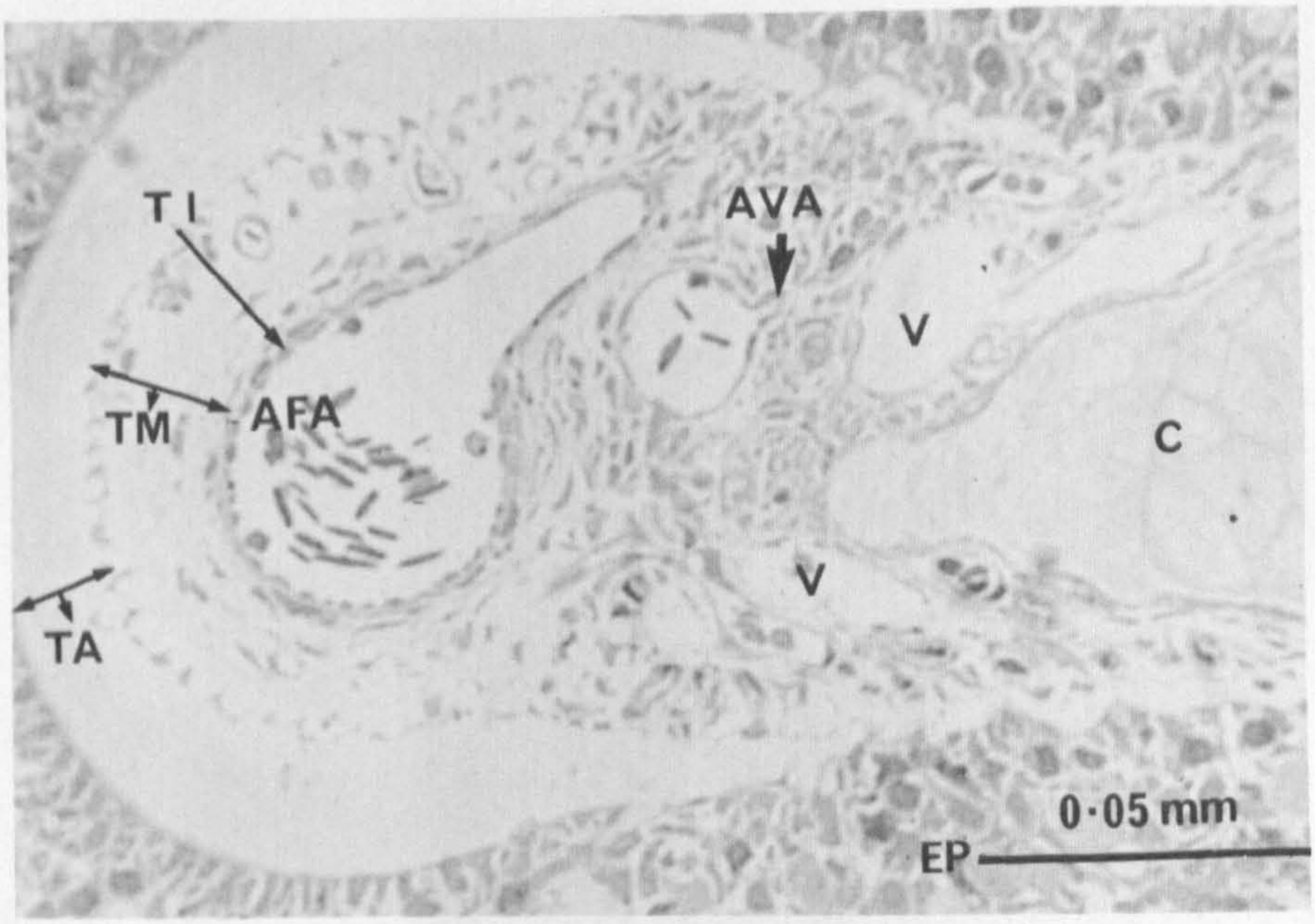

FIG. 3. Part of a transverse section of a gill filament showing wall of the afferent filament artery (AFA). The arrows show layers of AFA's wall and thick arrow indicates the arterio-venous anastomoses (AVA). Tunica intima (TI); tunica media (TM); tunica adventitia (TA); cartilaginous gill ray (C); epithelial layer (EP).

Each of the gill arches contain afferent and efferent branchial arteries (Fig. 1). The afferent branchial artery gives off an afferent filament artery to each gill filament of the two hemibranchs. Correspondingly, the efferent branchial artery receives efferent filament arteries from the gill filaments.

In each of the gill filaments, the afferent filament artery lies close to the cartilaginous gill ray which is found on the afferent side of each filament. The efferent filament artery is located at a distance from this supporting structure. However, the distance between the gill ray and the efferent filament artery varies at different levels of each filament (Fig. 2). The wall of the afferent filament artery is formed of tunica intima, tunica media and tunica adventitia (Fig. 3). The afferent filament artery gives off branches to each of the secondary lamellae. No valve which might serve to regulate blood flow in the lamellae has been seen at the origins of these two vessels (Fig. 4).

The outer surface of the efferent filament artery is not smooth but formed of the three usual layers (Fig. 5). It receives oxygenated blood from the secondary lamellae. As on the afferent side no valve was observed at the places where the blood channels from the secondary lamellae open into the efferent filament artery (Fig. 5).

THE ARTERIO-VENOUS PATHWAY

The arterio-venous pathway provides nutrition and oxygen to the gill filaments. It consists of the efferent filament artery, nutritive blood channels, central venous sinus, venules and the branchial veins. The most important part of the arterio-venous system is the central venous sinus. In *P. flesus* the central venous sinus is an extensive network of blood spaces lying between the cartilaginous gill ray

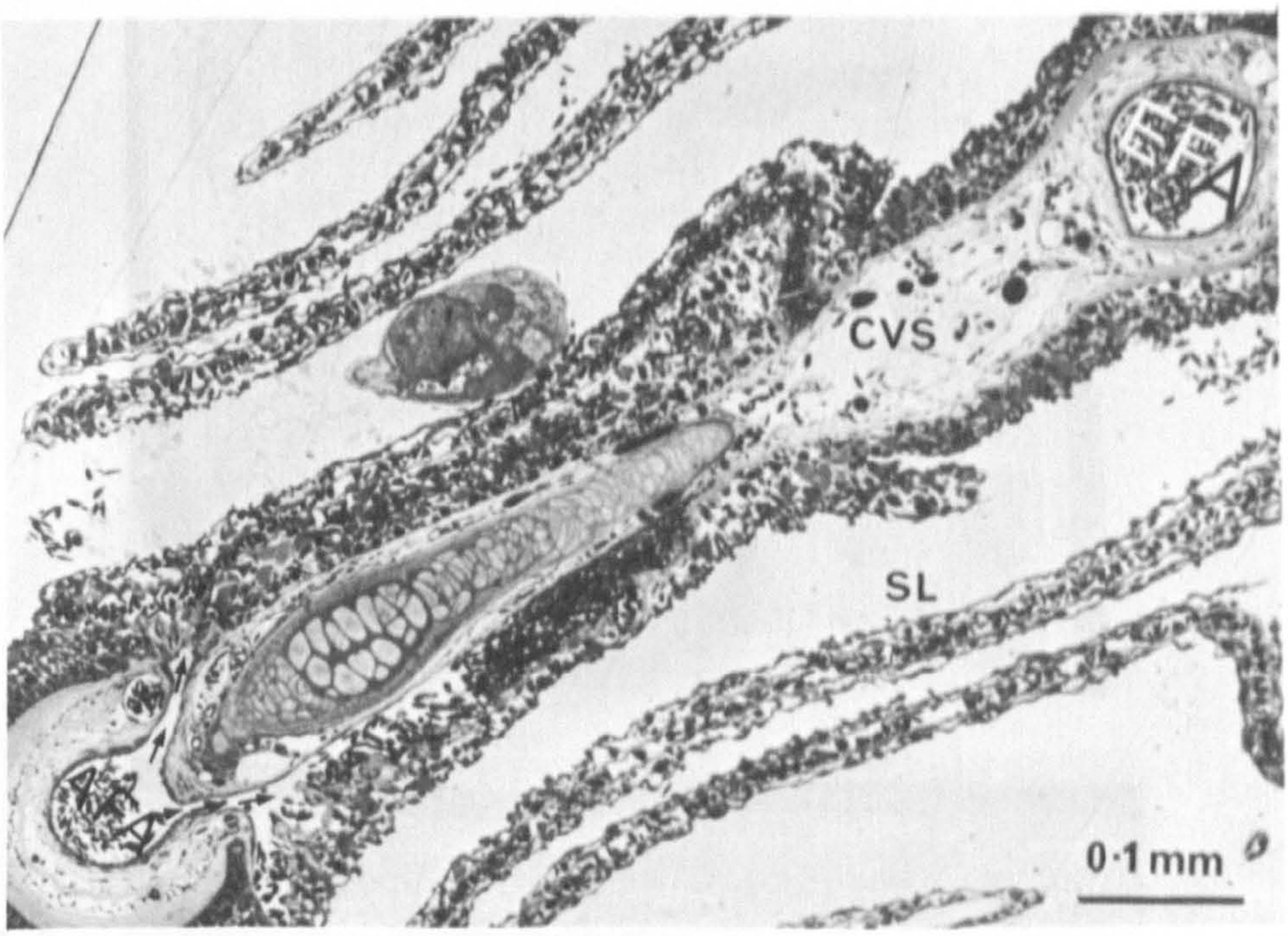

FIG. 4. Transverse section of gill filament showing the afferent filament artery (AFA) giving off two afferent secondary lamellar arteries as indicated by the arrows. Central venous sinus (CVS); cartilaginous gill ray (C); secondary lamellae (SL); efferent filament artery (EFA).

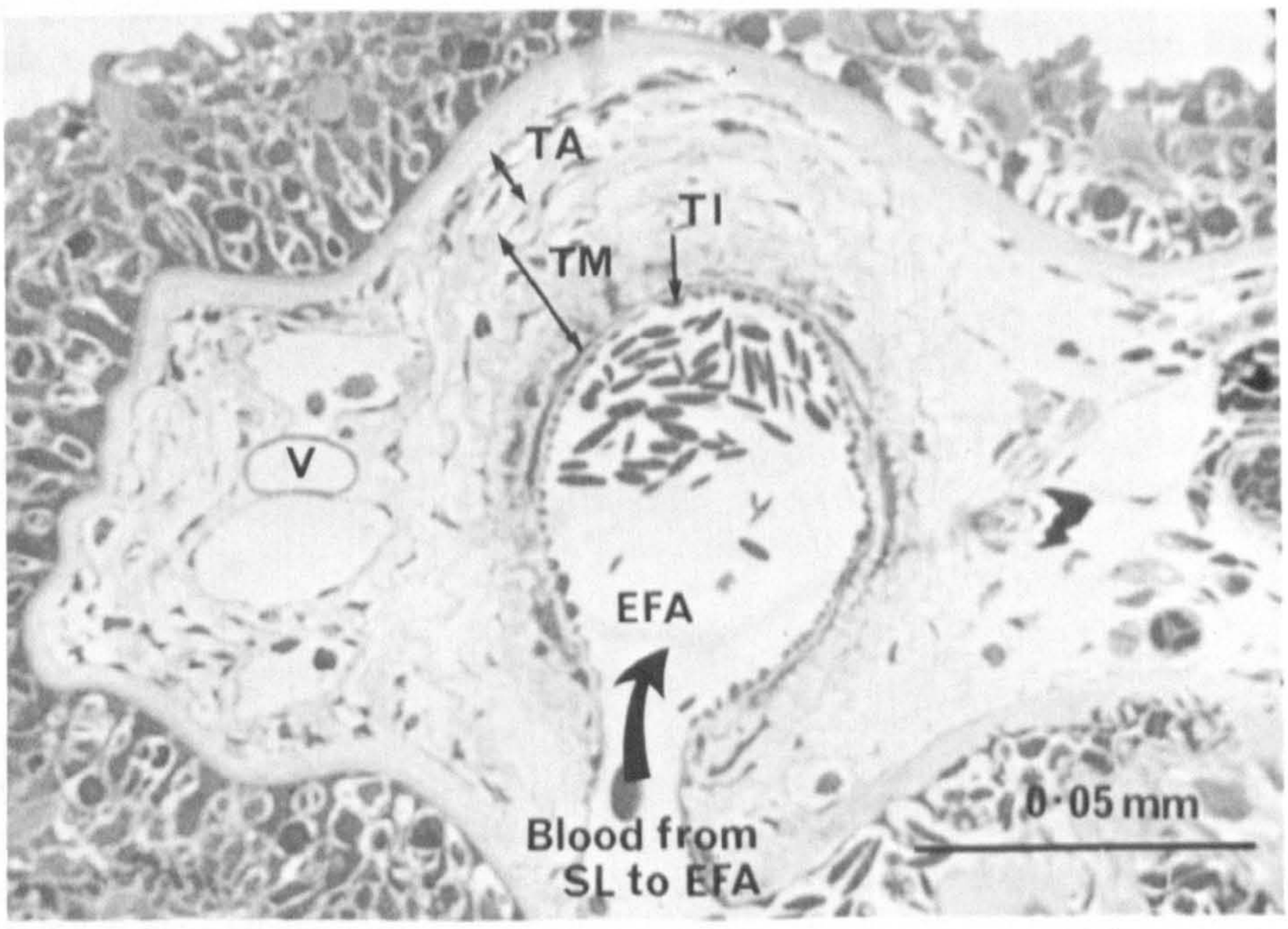

FIG. 5. Part of a transverse section through a gill filament showing the nature of the wall of efferent filament artery (EFA). Epithelial layer (EP); vein (CV); tunica intima (TI); tunica media (TM); tunica adventitia (TA).

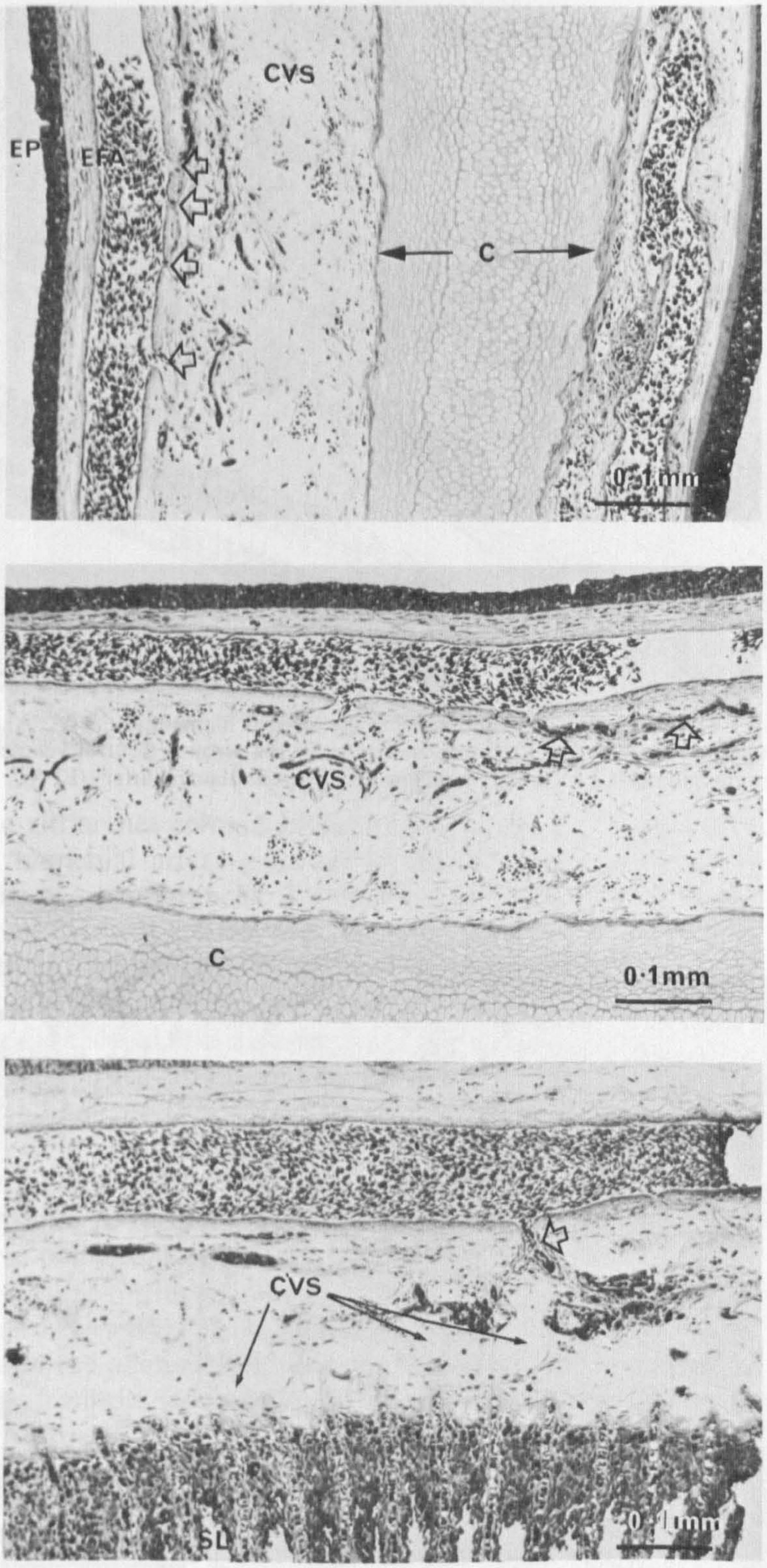

FIG. 6. Part of sagittal sections of a gill filament showing the central venous sinus (CVS) between the cartilaginous gill ray (C) and the efferent filament artery (EFA). Note the origin of the nutritive blood vessels as indicated by the open arrows. Afferent filament artery (AFA); secondary lamellae (SL); epithelial layer (EP).

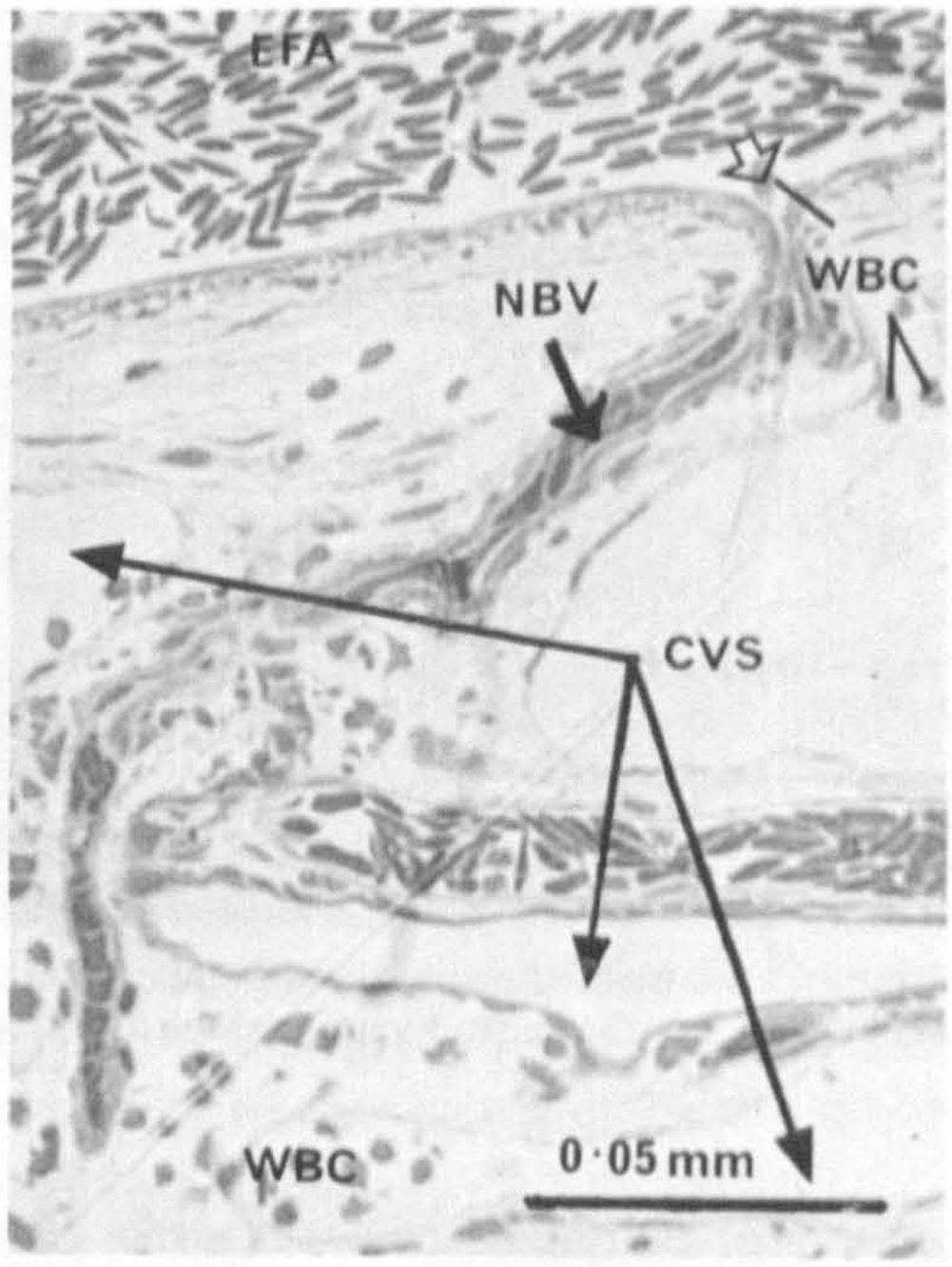

FIG. 7. Part of a sagittal section of a gill filament. The open arrow indicates a sphincter-like opening of the efferent filament artery (EFA) into the central venous sinus (CVS) through the nutritive blood vessels (NBV). White blood cells (WBC) are also shown.

and the efferent filament artery (Figs 6, 7). This vascular network is more extensive at the base than in the middle and the tip regions of the gill filaments (Fig. 2).

The efferent filament arteries communicate with the central venous sinus through sphincter-like structures (Figs 6, 7). At sites in each efferent filament artery there arise many nutritive blood channels which traverse the inner-core of the gill filaments. The central venous sinus in the gill filament of *P. flesus* is divided into two functional compartments. One compartment is mainly filled with plasma, red blood corpuscles (RBC) and a few white blood cells (WBC) and the other is dominated by WBC and a few RBC (Figs 8, 9).

In some sites the central venous sinus and veins do not contain blood corpuscles. Arterio-venous anastomoses have also been observed towards the afferent side of the filament (Fig. 3) with a secondary artery which contains RBCs but appears to be separate from the AFA; a direct connection between afferent filament artery and this vessel has not been observed.

IV. DISCUSSION

The existence of two independent vascular pathways in fish gills has been a matter of controversy since their description by Müller (1839) and Riess (1881). The present findings confirm the existence of two independent vascular pathways in the gills of *P. flesus*. The arterio-arterial pathway (respiratory) is of a typical teleostean type (Hughes & Grimstone, 1965; Vogel, Vogel & Schlote, 1974; Laurent & Dunel, 1976). Steen & Kruysse (1964) described the shunting of blood from the afferent to the efferent filament artery through the central venous sinus.

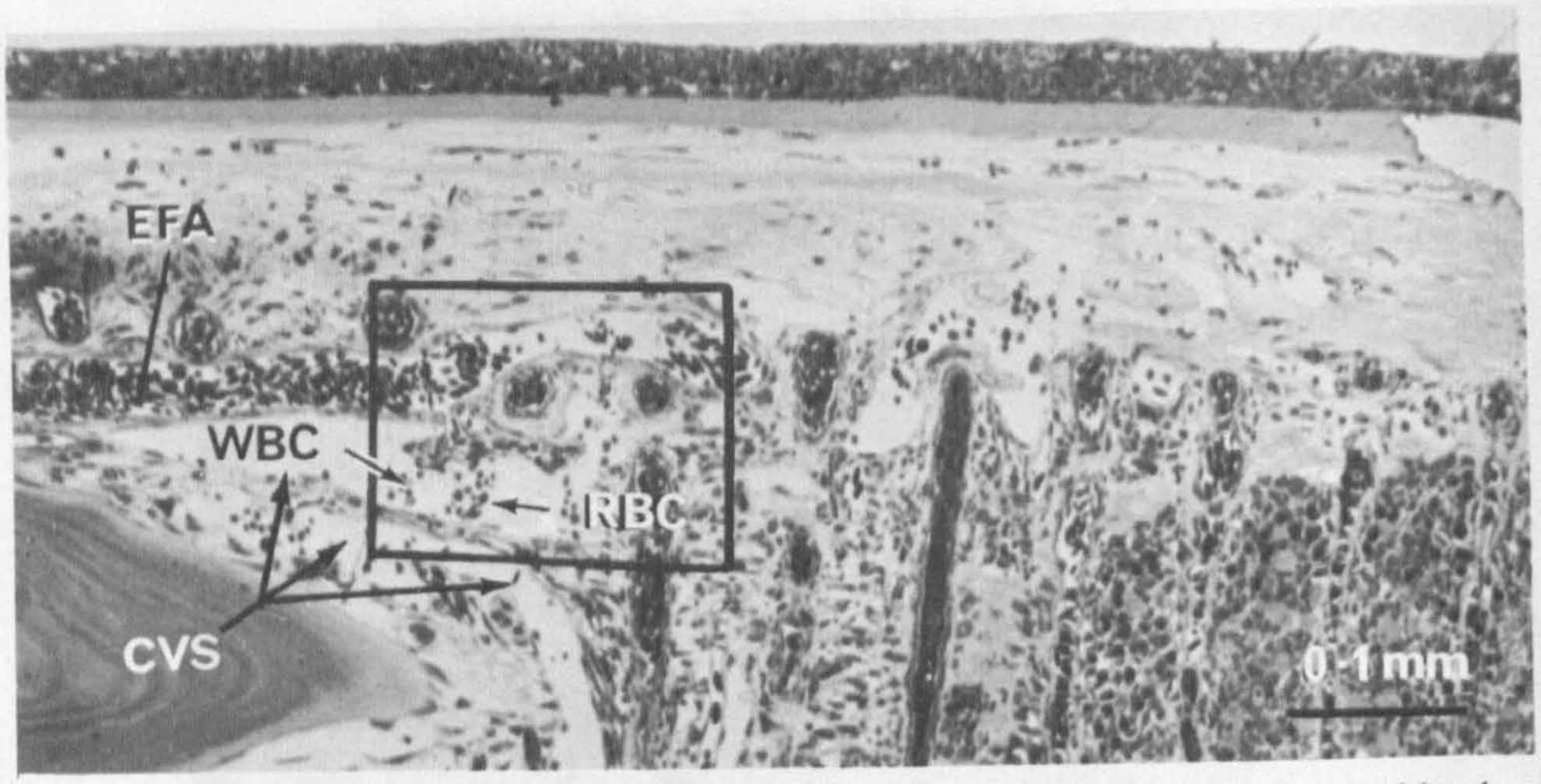

FIG. 8. Part of a sagittal section of a filament showing the difference in number of white blood cells (WBC) and red blood cells (RBC) in the central venous sinus (CVS) and efferent filament artery (EFA).

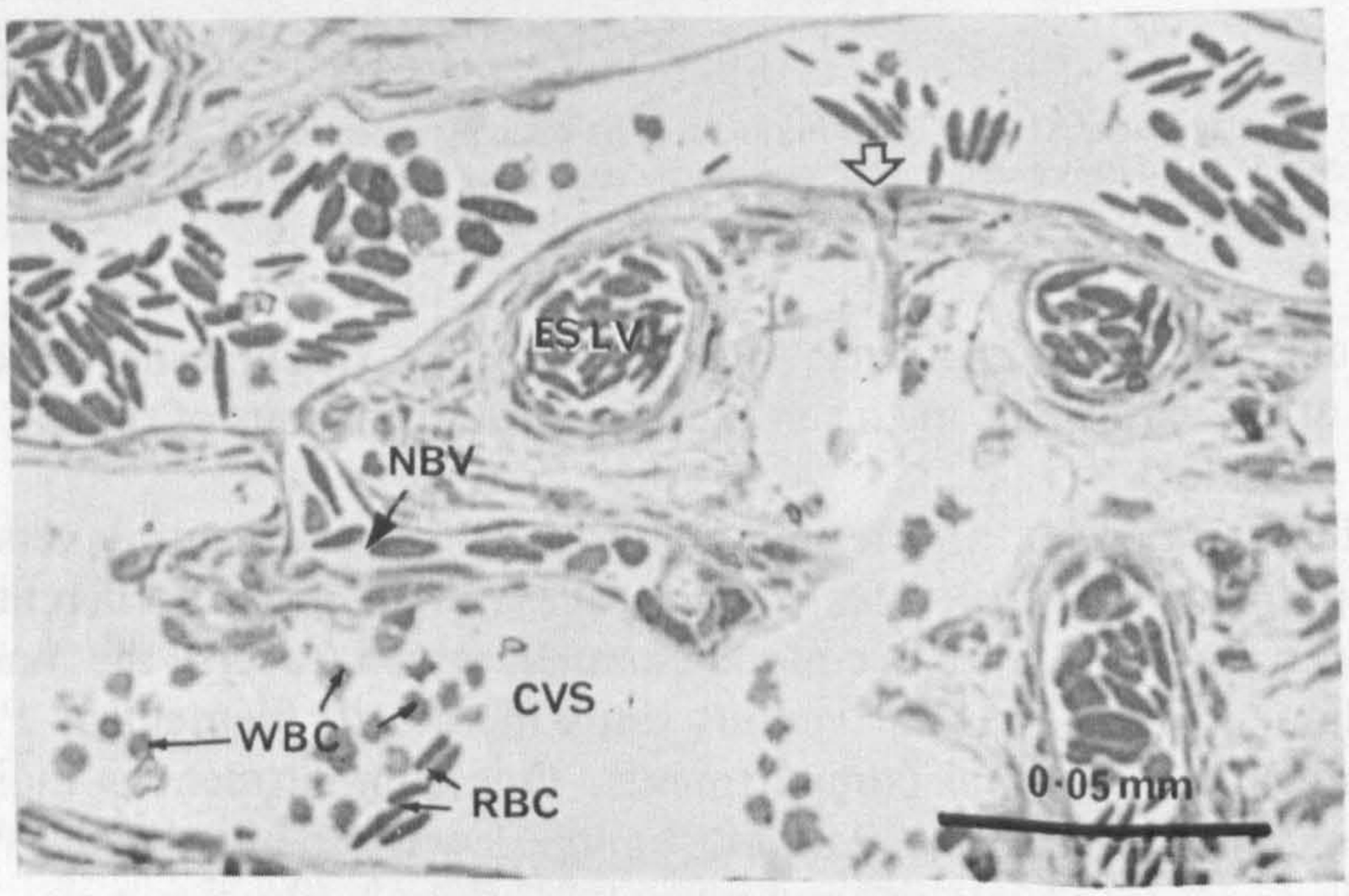

FIG. 9. Enlargement of area limited by black rectangle in Fig. 8. The open arrow indicates a small opening which mainly allows WBC but few RBC to pass through into CVS. Also shown are nutritive blood vessels (NBV) and efferent secondary lamella vessels (ESLV).

However, the presence of such a direct shunt has been doubted on both morphological and haemodynamic grounds (Hughes, 1972; 1979). The present study confirms the absence of such a direct pathway in *P. flesus* but evidence has been obtained for the passage of blood from the efferent filament arteries to the central venous sinus through small nutritive blood vessels. The origins of these vessels from the efferent filament arteries are guarded by sphincter-like structures which may help in the regulation of blood flow to the central venous sinus.

The view that separate vascular pathways may exist in fish gills through which few, if any, erythrocytes circulate has been suggested. These include the intra-epithelial 'lymphoid' spaces (Hughes & Wright, 1970; Hughes, 1980*a*) and an independent lymphatic system connected to the central venous sinus (Richards

& Fromm, 1969). Several morphometric studies have indicated that the heamatocrit value of different vascular pathways within the gills may also vary (Hughes, 1979, 1980*b*). Scanning electron micrographs (Vogel, Vogel & Schlote, 1974; Vogel, Vogel & Pfautsch, 1976) have shown that arterio-venous anastomoses between filament arteries (mainly efferent) and the central venous sinus are guarded by endothelial cells with finger-like projections into the vessel lumen and it has been suggested that such structures would restrict the flow of red cells into the CVS (Hughes, 1980*b*). Furthermore, plasma might pass directly through the endothelium as has been suggested for other parts of the fish circulatory system (Soivio & Hughes, 1978). The existence of an independent vascular system largely devoid of red blood cells may be a common feature of fish gills. In the flounder, the presence of such a compartment filled with white blood cells may be a special adaptation related to their habitat in the mud of estuaries and thus enable them to combat bacterial and fungal infections as has also been suggested for the phagocytic white cells in the lymphoid spaces of the secondary lamellae. The presence of such cells within the epithelium of the secondary lamella could form a defence mechansim similar to that provided by the alveolar macrophages in the mammalian lung (Hughes, 1980*a*).

The main supporting structure within the gill filaments of teleosts is provided by the gill ray which is situated towards the side of filament along which the afferent filament atery is found. The efferent side has no such support and the presence of the central venous sinus filled with plasma between the cartilaginous ray and the efferent filament artery may act as a hydroskeleton to provide additional support for this part of the filament.

It has also become clear from both physiological and morphological studies that the condition of the vascular circulation in fish gills is sensitive to environmental conditions. Consequently the precise conditions under which fixation of gill material is carried out can affect the appearance of the vascular pathways. Although the present description is based upon several specimens which were fixed in similar conditions, the possibility that the nature of the pathways observed could change cannot be ignored.

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